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REVIEW ARTICLE

Micronucleus assay of Buccal mucosa: a Useful noninvasive approach in Screening of Genotoxic nuclear damage

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ABSTRACT
Tobacco consumption was known in association of oral cancer. The present study was aimed to reviewing the studies related to influence of smoking on the buccal mucosa cells by the micronucleus assay. It was designed according to the guidelines of the 2009 Reporting Items for Systematic Reviews and Meta-Analysis. Relating to 26 articles which reviewed in the present study, it seems buccal cell micronuclei have been identified as useful biomarker in clinicopathologic investigations. Simplicity, accuracy, multipotentiality, being non-invasive and large tissue applicability of the MN technology made it attractive in early detection of oral cancer and will ensure a key role in the evaluation of mutagenicity and primary prevention in the future.
Keywords: *Micronucleus, smoking, oral cancer, screening*

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INTRODUCTION

Oral cancer is one of the most debilitating diseases afflicting mankind. It is a known fact that habits such as use of tobacco in various forms, alcohol consumption, pan chewing and use of commercial pan products are associated with increased risk of development oral cancer. The carcinogenic effect of the above mentioned habits may be related to inducing genotoxic effects on oral mucosal cells [1,2,3]. Consumption of tobacco in various forms constitutes one of the most important etiological factors in initiation of oral cancer. It has been shown that there is a dose-response relation between smoking and development of oral cancer [4-7].Tobacco is one of the strongest carcinogens, responsible for development of different types of cancers [8].

In developing countries like India, it is mainly consumed in two forms: smoked tobacco products and smokeless tobacco [9,10]. Regular consumption of tobacco in any form (chewing or smoking) has been strongly associated with cancers of the mouth, pharyngeal cavity, and upper digestive tract [11].Cigarette smoking, chewing of tobacco and their derivations are the major risk factors of oral cavity cancers(12).The smoking is a complex mixture of different type substances that are with a genotoxic and a carcinogenic effect on the oral epithelial cells [13], such as polycyclic aromatics hydrocarbons, aromatics amines, nitrosamines, heavy metals, poisonous gases and pesticide residues [14].Some tobacco specific nitrosamines, such as N-nitrosonor nicotine are potent carcinogens [15]. These materials activate in different tissues, which cause the DNA adducts products. The time influence on DNA adducts has controversial results [16].

Many different forms of smokeless tobacco are used in the world. An interesting kind of smokeless tobacco (Maras Powder) is used commonly in lieu of cigarettes in the South-Eastern region of Turkey.. This powder is prepared from *Nicotiana rustica* L. The dried leaves of the plant are powdered, then mixed

with ash by hand. A small amount of this mixture (~1 g) is applied to the lower lip mucosa for 4-5 min and then is spat out. This procedure is repeated many times during the day. Pharmacological studies have shown that this kind of smokeless tobacco is a form of buccal nicotine use [17].

The primary sites for occurrence of oral cancer include the buccal mucosa, tongue, alveolus, palate, lip and the floor of the mouth [18].

Oral cancer is often diagnosed at an advanced stage because of the lack of early diagnostic markers, and therefore, the survival rate is markedly reduced despite the best available treatment options [2]. There exists today a need to identify biomarkers of oral cancer and their association with tobacco usage [19] .The mouth is the only body site that permits viewing with the naked eye the ravages of smoked and smokeless "chewing" tobacco. For a given patient, it is often possible to view in the mouth during a clinical examination normal tissue, premalignant lesions (e.g., leukoplakia) and malignant tumors [20, 21].

The changes in buccal mucosa of tobacco chewers and cigarette smokers were demonstrated by micronucleus assay [22, 23]. When the focus of today's research is to determine early genotoxic changes in human cells, micronucleus (MN) assay provides a simple, yet reliable indicator of genotoxic damage (1).About 25 years ago, Stich *et al.*(24) introduced a method for micronucleus assay in exfoliated buccal mucosa cells. These structures are the result of chromosomal alterations [24-26]. Micronuclei originate from chromosome fragments or whole chromosomes that lag behind at anaphase during nuclear division [27,28].A micronucleus (MN) is a small extra nucleus separated from the main one, generated during cellular division by late chromosomes or by chromosome fragments. It is a microscopically visible round to oval cytoplasmic chromatin mass in the extra nuclear vicinity. They are induced in cells by numerous genotoxic agents that damage the chromosomes [1].

Micronucleus assay is a well validated method for testing genotoxic effects of various agents. It is the only biomarker that allows the simultaneous evaluation of both clastogenic and aneugenic effects in a wide range of cells [29]. Cytogenetic biomarkers, such as micronuclei in peripheral blood or oral mucosa, are widely used for evaluation of exposure to genotoxins or carcinogens [8].MN is induced in oral exfoliated cells by a variety of substances, including genotoxic agents and carcinogenic compounds in tobacco, betel nut, and alcohol [1]. It has been believed that the number of micronucleus is related to increasing the effects of carcinogens. The important point is that, this event has happened before clinical symptoms of cancer appear [30]. To further add, as it is applicable to interphase only, micronucleous assay is the best indicator of mitotic interference and chromosomal mutations or breakages [31]. Oral cytopathology is a simple technique that is non-aggressive, relatively painless, economical procedure and readily accepted by the patient. It is used to obtain cells from the oral epithelium [13]. No doubt, the collection of buccal cells is the least invasive method available for measuring DNA damage in humans, especially in comparison to obtaining blood samples for lymphocyte and erythrocyte assays, or tissue biopsies [31]. Since 1937 micronuclei have been regarded as indicators for genotoxic exposure. Clinical studies show that the determination of the MN rates in different cytological preparations can be reproducible. The loss of chromatin in the main nucleus due to a mutagenic exposition, contributes to the formation of micronuclei [32].

MN assay in oral exfoliated cells can be used as a simple reliable marker to assess the genotoxicity and for the early diagnosis of premalignant and malignant lesions [33, 34]. Investigations on MN frequencies support the widely accepted assumption that MN are a product of early events in human carcinogenic processes, especially in oral regions, especially because they are virtually absent in unexposed mucosa [31].

The present study was conducted with the aim of evaluating and gathering the studies that assessed the influence of smoking and smokeless tobacoo on the buccal mucosa cells by the micronucleous assay technique.

MATERIAL AND METHOD

Data sources

This study was designed according to the guidelines of the 2009 Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement [37]. The literature searches were done using short-string Boolean-based methodologies. Different Boolean search phrases were applied. These are denoted within <> brackets. The search phrase "operators" are presented in capital letters (e.g., <buccal cells AND smoking>). Various search terms were incorporated in the searches [e.g., "buccal cells," "smoking," "tobacco," "snuff,","micronucleous assay", "cigarette" and word variants (e.g., "smoke" where the dollar sign is used to identify all variants of the word)]. It was limited to prospective studies carried out up to September 2015, investigating the effects of smoking and smokeless tobacco on buccal mucosa cells.

All searches were free of bias and were unrestricted (i.e., excluded limits); thus, the explorations included all (a) fields (e.g., author and title), (b) languages, (c) dates, (d) subjects (e.g., humans and animals), and (e) database subject subsets (e.g., cancer and tobacco).

The first literature search was done using PubMed at http://www.ncbi.nlm.nih.gov/. This exploration identified 7792 publications addressing <micronucleous assay>. The results of other searches were as follows: <buccal mucosa cells > yielded 8387 articles, <buccal cells AND smoking> yielded 340 articles, <buccal cells AND micronucleous assay> yielded 250 articles, <buccal cells AND micronucleous assay AND smoking > yielded 71 articles and <buccal cells AND micronucleous assay AND smoking AND smokeless tobacco> yielded 7 articles. The literature searches were extended by using the "Related Articles" link to articles recovered with PubMed. Thereafter, a search was done of the literature references cited in these articles to identify additional writings that reported the results of research defining buccal cell changes associated with tobacco use.

Study selection

Inclusion criteria [35, 37]

Original studies were included if they met the following inclusion criteria:

a) Being a prospective clinical study;

b) Investigating the effect of smoking and smokeless tobacco on buccal mucosa cells;

c) Investigating buccal mucosa cells by the method of micronucleous assay technique;

Exclusion criteria

Exclusion criteria were:

a) Non-clinical studies (experimental and basic studies);

b) Observational or retrospective studies;

c) Duplicate reports or secondary or post hoc analyses of the same study population;

d) Lack of sufficient information on baseline.

Then, Two reviewers examined every article separately to minimize the possibility of duplication, investigating reviews, case studies and experimental studies At this juncture, all articles were assembled and arranged in reverse chronological order. The articles were read, and off-topic articles were excluded. For example, rejected articles included those that reported research of cells other than buccal cells (e.g., oral leukocytes). Likewise, reports of investigations of buccal cell changes that were not associated with tobacco were excluded (e.g., radiation).

Data extraction

Eligible studies were reviewed and the following data were abstracted:

1) Subject; 2) Author's name; 3) year of publication; 4) samples; 5) country; 6) Method and material; 7) conclusion

Then, all the information from included articles was extracted into the Table 1.

Table 1: The information from reviewed articles

NO.	Subject& No of ref	Samples country	Method and material	Conclusion
1	The micronucleus assessment of buccal mucosa: a noninvasive method in screening of smokers potentially exposed to oral cancer [40]	Iran 48	Buccal mucosa smear was obtained after Papanicolaou staining, 1000 cells of each sample were inspected under light microscope and cells with micronucleus were counted	The average frequency of micronucleus was higher in smokers compared to non-smokers. this noninvasive assessment may help in early diagnosis of oral cancer
2	Comparative Study of Genotoxicity in Different Tobacco Related Habits using Micronucleus Assay in Exfoliated Buccal Epithelial Cells [1]	Mangalore 135	Three smears from the lateral left border of the tongue were processed for Feulgen staining and micronucleus frequency was evaluated.	tobacco in any form is genotoxic especially for smokers who are at higher risk and micronucleus assay can be used as a simple yet reliable marker for genotoxic evaluation
3	Assessment of genotoxic and molecular mechanisms of cancer risk in smoking and smokeless	India ??	micronucleus frequency was measured in peripheral blood and buccal mucosa exfoliated cells	MN assay showed both genotypes are associated with increased risk of cancer.

	tobacco users [41]			
4	Micronuclei as prognostic indicators in oral cytological smears: A comparison between smokers and non-smokers [19]	India 50	Buccal smears of all participants were taken using cytobrush and stained with standard Papanicolaou's (PAP) stain	The genotoxic effects of tobacco smoke cause chromosomal damage in the epithelial cells of the oral mucosa and Reflect in the increased micronuclei in smokers. This is present even in the absence of clinically evident changes.
5	Assement of cytogenic damage in form of micronuclei in oral epithelial cells in patients using smokeless and smoked form of tobacco and non-tobacco users and its relevance for oral cancer(42)	India 100	Staining with papanicolaou and micronucleus assay	MN assay showed that using smokeless and smoked tobacco is associated with cytogenic and genotoxic effect.
6	Potential Uses, Limitations, and Basic Procedures of Micronuclei and Nuclear Abnormalities in Buccal Cells(38)	Mexico	Review article	Micronucleus technique is reliable, fast, relatively simple, cheap, and minimally invasive and causes no pain. So, it is well accepted by patients; it can also be used to assess the genotoxiceffect derived from drug use or as a result of having a chronic disease.
7	Genotoxicity assessment in smokeless tobacco users: a case-control study [43]	India 170	evaluate the genotoxic effects of tobacco use by analyzing the cytogenetic end points such as chromosome aberrations in peripheral blood and micronucleus in peripheral blood and buccal cells	MN assay in this study might be helpful in creating awareness on the hazards of the smokeless tobacco products among the global population as a whole for those who chose such products as a cheap alternative to tobacco smoke.
8	MICRONUCLEUS INVESTIGATION IN EXFOLIATED BUCCAL CELLS AMONG TOBACCO CHEWERS/ SMOKERS AND CONTROLS [29]	India 115	micronucleated cells (MNC's) were Evaluated from exfoliated buccal mucosal cells.	Two confounding factors i.e. age and duration of exposure showed significant association while the third confounding factor i.e. alcohol intake did not show any significant association with the MN frequency
9	A comparative study of oral epithelium in tobacco and alcohol consumers in central Rajasthan population [44]	India 800	Staining with papanicolaou technique and micronucleus assay	Microscopically nuclear changes like micronucleous are a useful tools in early diagnosis of the oral carcinoma.
10	Micronucleus assay of buccal mucosa cells in smokers with the history of smoking less and more than 10 years(36)	Iran 63	The presence of micronucleus in all subjects and the mean percentage of micronucleus in nuclei were determined	The mean number of micronuclei in buccal mucosa cells of the nonsmokers was significantly lower than that of the smokers
11	Comparative study of oral mucosa micronuclei in smokers and alcoholic smokers [45]	Brazil 51	Three smears from the lateral left border of the tongue were processed for Feulgen staining	The action of genotoxic agents (tobacco and alcohol) causes alterations in the frequency of micronuclei and metanucleated anomalies
12	A cytopathological study of the effect of smoking on the	Iraq 75	The oral health status was evaluated by using the plaque, gingival, calculus	The micronucleus assay detected by Pap stain is a useful biomarker to detect the people

	oral epithelial cells in relation to oral health status by the micronucleus assay [13]		indices in addition to the amalgam and composite restorations.	at high risk of oral mutations due to the harmful effect of the smoking, the calculus and plaque indices, in addition to the amalgam restorations
13	Evaluation of micronuclei in tobacco users [46]	India 75	Staining with papanicolaou and micronucleus assay	A positive correlation is found between increased micronucleous frequency and tobacco-users habits. So MN assay can be used as a biomarker of genotoxicity.
14	Micronuclei assay of exfoliated oral buccal cells: means to assess the nuclear abnormalities in different diseases(39)	India	Review article	Simplicity, accuracy, multipotentiality, and large tissue applicability of the MN technology made it attractive in the past and will ensure a key role in the evaluation of mutagenicity and primary prevention in the future.
15	The Human Micronucleus project on exfoliated buccal cells (HUMN(XL)): the role of life-style, host factors, occupational exposures, health status, and assay protocol [47]	Italy 5424	The HUMN(XL) project evaluates the impact of host factors, occupation, life-style, disease status, and protocol features on the occurrence of MN in exfoliated buccal cells	MN frequency increased in heavy smoking and decreased with daily fruit consumption
16	Evaluation of smoking genotoxicity in Turkish young adults [48]	Turkey 30	MN assay was performed on buccal mucosa, urothelial cells, and peripheric blood lymphocyte samples	MN assay showed cigarette smoking is a DNA damage causative agent on exfoliative buccal mucosa and urothelial cells and peripheric blood lymphocytes of young smokers, but it has most destructive effect on urothelial cells
17	Micronuclei frequencies in peripheral blood and buccal exfoliated cells of young smokers and non-smokers [8]	Bosnia and Herzegovina 87	MN assay of cigarette consumption in young smokers and to correlate results of cytogenetic analysis in peripheral blood lymphocytes and exfoliated buccal cells	Results of MN assay conducted in peripheral blood and exfoliated buccal cells are in significant positive correlation, indicating complementarities of those analyses
18	Genotoxic effects of waterpipe smoking on the buccal mucosa cells [49]	Egypt 206	examination oral smears of 128 adult male waterpipe smokers and 78 males who never smoked tobacco	The twofold increase in MN level is consistent with previous reports of MN in cigarette smokers
19	Smoking and Smokeless Tobacco-Associated Human Buccal Cell Mutations and Their Association with Oral Cancer—A Review [35]	USA	Systematic review	buccal cells are useful not only for characterizing the molecular mechanisms underlying tobacco-associated oral cancers but also as exfoliative cells that express diverse changes like micronucleus that offer promise as candidate biomarkers for the early detection of oral cancer
20	Can micronucleus technique predict the risk of lung cancer in smokers? [50]	Turkey 22	Spontaneous and radiation induced MN frequencies were evaluated in the two groups	radiation induced MN scores in peripheral blood lymphocytes of long term smokers do not predict the risk of lung cancer
21	Application of the micronucleus test to	India 50	examination an early cellular response to the	The difference in mean micronucleated cell count

	exfoliated epithelial cells from the oral cavity of beedi smokers, a high-risk group for oral cancer [18]		effect of beedi smoking by using the micronucleus assay on exfoliated cells from the buccal mucosa, palate and tongue of beedi smokers	between cases and controls was significant for buccal mucosa and palate, but not for tongue
22	Clastogenic effect for cigarette smoking but not areca quid chewing as measured by micronuclei in exfoliated buccal mucosa cells [51]	Taiwan 141	Micronuclei were scored on Feulgen/fast green-stained smear preparations of exfoliated cells obtained by scraping the surface of the buccal mucosa	Heavy smoking was positively associated with micronuclei frequency; areca quid chewing was negatively associated. A significant positive trend was shown for the relationship between micronuclei frequency and either daily cigarette consumption or cumulative smoking pack-years. .
23	Effect of smoking and aging on micronucleus frequencies in human exfoliated buccal cells [52]	Poland 120	the frequency of micronuclei (MN) in exfoliated buccal cells in 120 healthy individuals with relation to sex, age and smoking was investigated	MN assay showed Smoking has a significant effect upon basal DNA damage. Also, Higher frequency of micronuclei was observed for cells collected from female smokers than male smokers . Age and gender did not influence micronuclei frequency of nonsmokers
24	Induction of micronuclei by smokeless tobacco on buccal mucosa cells of habitual users [17]	Turkey 54	evaluated micronuclei in buccal mucosa cells of habitual Maras Powder users	MN assay showed the genotoxic effect of smokeless tobacco should be considered in addition to other known hazards
25	Cigarette smoking, intracellular vitamin deficiency, and occurrence of micronuclei in epithelial cells of the buccal mucosa [53]	USA 99	Methods used to measure folate, vitamin B12 levels, and the frequency of micronucleated cells in buccal mucosal cells gave reproducible results	MN assay showed the presence of vitamin B12 deficiencies in the buccal mucosal cells of smokers are associated with chromosomal damage in those cells
26	Quantitating the synergistic effect of smoking and alcohol consumption with the micronucleus test on human buccal mucosa cells [54]		The micronucleus test was applied to exfoliated cells of the buccal mucosa of four population groups: (A) non-smokers and non-drinkers of alcoholic beverages, (B) non-smokers but alcohol drinkers, (C) smokers but non-drinkers, and (D) smokers and drinkers	Whether the strong synergistic effect between smoking and alcohol consumption, as seen by the frequency of micronucleated buccal mucosa cells, is related to their synergistic effect in the induction of oral cancers is an intriguing but open question

RESULT

From 26 studies that were evaluated in this study, 10 cases (number 2,3,5,7,8,18,19,20,21 and 23 of table 1) have considered the effect of other form of tobacco (such as chewing tobacco) lonely or with smoking tobacco on the micronucleus frequency. Most of the mentioned studies have connected the consumption of all kind of tobacco with increase in micronucleated cells frequency and in some cases (No 10) even with increase in number of micronuclei in each cell.

Six studies (No 1, 4, 13, 16, 17, 22) have mentioned the effect of smoking tobacco on increasing the number of micronucleus and have reported the significant relation between them. Three studies (No 9, 11, 26) have evaluated the effect of coincident consumption of cigarette and alcohol on the number of micronucleus and have considered both of them were affective on increasing the number of micronucleus. One of the studies has considered that coincident consumption of alcohol cigarette has a

synergic and fortifier effect on increasing the number of micronuclei and finally increasing the risk of cancer. In other evaluated studies in present study (No 12, 15, 23, 25) other variables such as life style, oral and overall health status, personal exposures, vitamin deficiency, aging and sex also were evaluated in addition to the smoking of cigarette and some of them have reported the significant relation.
Some studies (No 8, 10, 21) have evaluated the effect of smoking time on increasing the number of micronuclei and each three studies have indicated that increasing in number of micronuclei is associated with increasing in smoking time or smoking pack-day.
Also, some studies have evaluated the micronucleus in peripheral blood in addition to the buccal mucosa cells and have reported the results in both buccal cells and peripheral blood with high correlation (No 16, 17).
Among the evaluated studies in this study, two review article are appeared that were performed in 2006 and 2014 (No 6, 19) and have exhausted more often to correctness of the buccal cells usage in early detection of genetic changes lead to cancer. In the results they have believed that usage of these cells in evaluation the risk of malignancy with nuclear changes of buccal cells such as micronucleus assay is useful and they have indicated the micronucleus assay as a biomarker in this field.

DISCUSSION

A total of 26 articles were evaluated by micronucleus assay that has been done to determine if buccal cell changes were associated with the consumption of tobacco. An analysis of these documents revealed that different tests were used. In this literature review, we have identified shortcomings should be acknowledged and may provide guidance in undertaking future investigations. Different assays have been used successfully for documenting tobacco-associated chromosomal and genetic changes in buccal cells with most frequently of"buccal cell micronuclei assay" (Table 1). The popularity of this assay has prompted the authors to review this assay as applied to the study of buccal cells .
According to table 1 some studies like Sharma *et al.* [44] have evaluated the effect of coincident consumption of cigarette and alcohol on the number of micronucleus and have considered both of them were affective on increasing the number of micronucleus. One of the studies has considered that coincident consumption of alcohol cigarette has a synergic and fortifier effect on increasing the number of micronuclei and finally increasing the risk of cancer.
In some studies other variables such as life style, oral and overall health status, personal exposures, vitamin deficiency, aging and sex also were evaluated in addition to the smoking of cigarette and some of them have reported the significant relation. Konopaka *et al.* [52] have reported that MN assay showed Smoking has a significant effect upon basal DNA damage. Also, higher frequency of micronuclei was observed for cells collected from female smokers than male smokers. Age and gender did not influence micronuclei frequency of nonsmokers. Saeed *et al.* [13] also have assessed that the micronucleus assay detected by Pap stain is a useful biomarker to detect the people at high risk of oral mutations due to the harmful effect of the smoking, the calculus and plaque indices, in addition to the amalgam restorations. Bonassi *et al.* [47] have reported MN frequency increased in heavy smoking and decreased with daily fruit consumption [47].
Caplash *et al.* [18], Naderi *et al.* [36] and Suhas *et al.* [49] have evaluated the effect of smoking time on increasing the number of micronuclei and each three studies have indicated that increasing in number of micronuclei is associated with increasing in smoking time or smoking pack-day. Naderi N.J. *et al.* [36] have concluded that the mean number of micronucleus of buccal mucosa cells in smokers who smoked more than 10 years was higher than smokers who smoked less than 10 years. Increasing the smoking's duration could heighten the frequency of micronucleus number; however, the difference was not significant.
Zamani A.G. *et al.* [48] and Haveric A. *et al.* [8] have evaluated the micronucleus in peripheral blood in addition to the buccal mucosa cells and have reported the results in both buccal cells and peripheral blood with high correlation. Zamani *et al.* [48] have reported that MN assay showed cigarette smoking is a DNA damage causitive agent on exfoliative buccal mucosa and urothelial cells and peripheric blood lymphocytes of young smokers, but it has most destructive effect on urothelial cells.
Today, buccal cell micronuclei assays are being used that differ significantly among different laboratories. The observed variance from prescribed methods discloses that a standardized protocol has not been universally accepted. Thus, there exists a need to review the micronuclei assay protocols that are being used .
Therefore, there remains a need to establish a standardized buccal cell assay that is rapid, inexpensive, quantitative, reproducible, technologically simple, and applicable for monitoring longitudinal studies with

relatively large cohorts of subjects who have been identified as being at high risk for developing oral cancer [35].

According to the research of Proia *et al.* [35], the studies have identified a positive association of tobacco consumption with buccal cell mutations. Consequently, buccal cell mutations are useful biomarkers that are a useful adjunct to current and emerging clinical screening procedures.

Oral cancer is a lifestyle-related cancer with tobacco as a primary factor. Oral cancer, however, is the result of a long-standing process that progresses over several decades. Behavior intervention to quit smoking may be greatly facilitated if the subject is aware of risk markers that have been identified in the clinically normal oral mucosa. Thus, the identification of risk markers of oral cancer may serve as an aid in smoking cessation counseling [35].

Kashyap *et al.* [39] have indicated that although many studies have consistently shown a statistically significant increase in the buccal cell MN frequency in human populations exposed to genotoxic agents, or a decrease as a result of micronutrient supplementation or chemoprevention, the magnitude of changes is usually relatively small. Different confounding factors influencing the MN frequency in peripheral lymphocytes, such as gender, age, and lifestyle habits have been considered for the buccal cell MN assay.

Also, they have added despite the considerable potential of the buccal MN assay for biomonitoring, the diversity of possible methodologic variables, and their impact on assay performance, could hinder consistency among laboratories with regard to measuring the effects of dietary, lifestyle, and genetic factors [39].

Torres-Bugarín *et al.* [38] have stated that conditions that facilitate MN assay use include the fact that it can be easily done and has aminimally invasive sampling procedure. The most common application of the MN assay in buccal cells concerns occupational and environmental exposure togenotoxic agents, in this field, a diversity of biomarkers exist. In toxicology, is recognized the fact that more than one test in necessary in order to demonstrate a causal effect of a determinant pollutant .

It can also provide valuable information on the stage of progression of some degenerative diseases. However, the role of the MN assay and its applicability in human populations needs to be established and to be more clearly defined [38].

CONCLUSION

The MN assay in buccal cells has been widely applied worldwide and its use has been increasing in the last decade. Buccal cell micronuclei have been identified as useful biomarker in clinicopathologic investigations, and that a high-throughput assay can be developed for screening smokers for the early detection of oral cancer .

Simplicity, accuracy, multipotentiality, being non-invasive and large tissue applicability of the MN technology made it attractive in early detection of malignancy and will ensure a key role in the evaluation of mutagenicity and primary prevention in the future.

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