A cytopathological study of the effect of smoking on the oral epithelial cells in relation to oral health status by the micronucleus assay

Saeed H. Saeed B.D.S. [(1)]
Wasen H. Younis B.D.S., M.Sc., Ph.D. [(2)]

ABSTRACT

Background: Micronucleus is a cytoplasmic fragment of DNA reported as a biomarker of cancer. It is a cytoplasmic chromatin mass formed in the basal cells layer of the epithelium. These fragments can form their own membrane. The aims of the study was to detect the micronuclei expression in the oral epithelial cells in cytopathological smears of the non-smokers' and the smokers' males, correlate the micronuclei expression in the oral epithelial cells with the oral health status variables, and evaluate the efficacy of the micronuclei assay to detect the subjects at high risk of oral mutations.

Materials and methods: This study was conducted on 75 males of (35- 40) years of age divided into 25 heavy smokers, 25 light smokers, and 25 non-smokers. A cytobrush was used to obtain the smears. The oral health status was evaluated by using the plaque, gingival, calculus indices in addition to the amalgam and composite restorations.

Results: There was a statistically significant difference in the micronuclei expression among the three groups. There was a strong correlation between the oral health status variables and the micronuclei expression in the non- smokers' group, for the Plaque index with (P-value =0.0005) and for the calculus index (P-value = 0.04). The smokers' group had a strong correlation with the amalgam restorations with (P-value =0.0005).

Conclusion: 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.

Key words: Cytopathology, micronucleus assay, smoking effects. (J Bagh Coll Dentistry 2012;24(3):67-70).

INTRODUCTION

The oral epithelial cells represent the preferred target site for the early genotoxic events induced by different types of agents entering the oral cavity [(1)]. The smoking is a complex mixture of different type substances that are with a genotoxic and a carcinogenic effect on the oral epithelial cells. These substances lead to the DNA damage and the nuclear anomalies formation. One of these a nuclear anomaly is the micronuclei formation [(2)].

Micronucleus is a cytoplasmic fragment of DNA reported as a biomarker of mutagenesis. It is a cytoplasmic chromatin masses that can form its own membrane. The micronuclei are formed in the basal cells layer. The micronucleus assay is used to detect the subjects at high risk of malignant transformations in their oral epithelial cells [(3)]. It has been extensively used to evaluate the extent of chromosomal damage in the human population exposed to the genotoxic agents in various occupational settings, in the environment, or as a consequence of the life style [(4)].

The micronuclei test is gaining an increased attention among researchers and laboratories in the field of environmental mutagenesis, and a number of published studies based on this biomarker are increasing rapidly [(5)].

Oral cytopathology is a simple technique that is non-aggressive, relatively painless, and readily accepted by the patient. It is used to obtain cells from the oral epithelium. It is the art and science of interpretation of the cells obtained from the oral cavity. The oral epithelial cells may be detached naturally or artificially as in scrubbing and the cytobrush sampling [(6)]. In the current study, micronuclei assay was sought to be used for the first time on Iraqi sample to evaluate its validity as a biomarker for the early detection of the oral epithelial cells with mutation in relation to the effect of the smoking as a very popular habit, on the oral epithelial cells from different keratinized and non-keratinized oral sites. The smoking is a complex mixture of different types of substances that have a mutagenic and carcinogenic effect on the oral epithelial cells. The smoking effect on the oral epithelial cells was evaluated in relation to the different oral health status variables. The oral health status variables include the plaque, gingival, calculus indices and the amalgam and the composite restorations.

(1) M.Sc. Student, Ministry of Heath
(2) Professor, Chairman of Oral Diagnosis Department, College of Dentistry, University of Baghdad.

MATERIAL AND METHODS

Seventy five males' volunteers aged (35-40) year attending to the oral diagnosis department/collage of dentistry/Baghdad University, the maxillofacial clinic in Al Hussein hospital, and specialized center for Dentistry in Karbala. The oral examination includes the determination of the types of restorations in each tooth by the application of the FS fraction of the DMFS index. Oral health status for each patient was assessed by using plaque, gingival and calculus index.

Oral smear should be obtained from normal mucosa by using the cytopathological brush. The oral sites include: - buccal mucosa, hard palate, gingiva and floor of mouth. The smears were transferred and spread onto the labeled, clean, dry glass slide. Each slide was labeled with the patient's name ,the site from which the sample obtained and the type of stain .For Pap stain method , the slides were fixed at once by 95% ethanol for 20 minutes, whereas, they were air dried for Giemsa stain method. Each oral site has two slides, the first one was stained by Pap stain and the second by Giemsa stain. Each patient has 8 slides and 1000 oral epithelial cells examined by the light microscope.

RESULTS

All the subjects of the study sample were with a positive expression of the micronuclei in different numbers (table1,2, and 3) (Figures1,2,3, and 4). There was a statistically significant difference in the micronuclei expression between the non-smokers and the light smokers, where (P= 0.031) in the floor of mouth stained by Pap stain. There was a statistically significant difference in the micronuclei expression between the non- smokers and the heavy smokers where (P = 0.0005) in both the floor of mouth and the gingiva, (P = 0.002) in the buccal mucosa, and (P= 0.004) in the palate stained by Pap stain. For the slides stained by Giemsa stain, floor of mouth, gingiva, and the buccal mucosa (P = 0.001), while palate was a non-significant difference (P=0.685). There was a statistically significant difference between the light smokers and the heavy smokers where (P = 0.0005) for all the oral sites stained by Pap stain, and floor of mouth stained by Giemsa stain only showed a highly significant difference (P=0.0005). Both gingiva and palate were with a (P=0.521), while buccal mucosa was (P = 0.59) for the slides stained by Giemsa stain for the micronuclei expression (table4).

There was a strong correlation between the oral health status variables and the micronuclei expression in the non- smokers' group, for the Plaque index with (P =0.0005) and for the calculus index (P = 0.04). Regarding the smokers' group, they had a strong correlation with the amalgam restorations with (P =0.0005). According to the multiple linear regression model indicated that the non-smokers' group was with (P =0.007) for the calculus index, while in the smokers' group (P = 0.006) for the amalgam restorations.

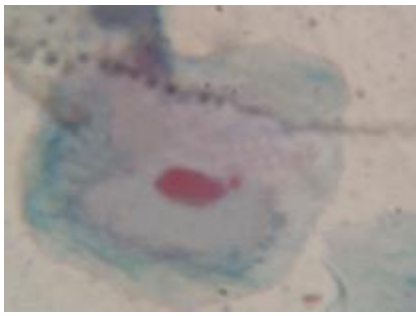

Figure 1: An oral epithelial cell with micronucleus stained by Pap stain at X100 oil emersion

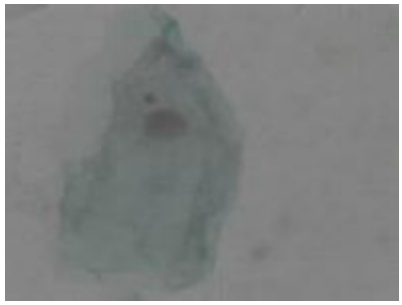

Figure 2: An oral epithelial cell with micronucleus stained by Giemsa stain at X100 oil emersion

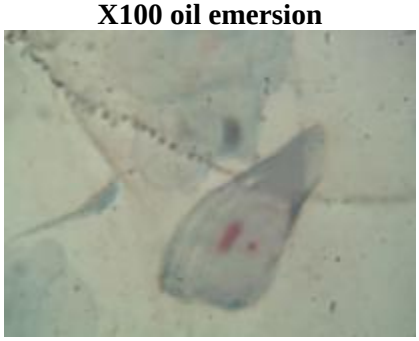

Figure 3: An oral epithelial cell with micronucleus stained by Pap stain at X40

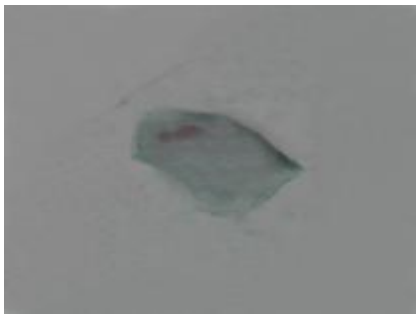

Figure 4: An oral epithelial cell with micronucleus stained by Giemsa stain at X40

DISCUSSION

The study results revealed that, there was a micronuclei expression in all the smears taken from the examined males, but in different proportions. The mean of the micronuclei expression in the non-smokers' group was (2.36) micronucleus in each 1000 oral epithelial cells. This was consistent with the baseline of the micronuclei expression in the healthy subjects which was (0.5-2.5) micronucleus per 1000 oral epithelial cells according to the results of [7] who studied the micronuclei expression in the oral epithelial cells from patients with cancer, pre-cancerous lesions, and healthy controls. They found an 11 fold increase in the micronuclei expression in the patients with cancerous oral lesions (p-value = 0.001) and (10.38) fold increase in the micronuclei expression in the patients with pre-cancerous oral lesions (p-value =0.002). The current study was also related to the oral health status variables and their effect on the oral epithelial cells in relation to the smoking by the micronuclei expression. In the non- smokers' group, there was a strong relation of the plaque index and the calculus index with the increase in the micronuclei expression in the oral epithelial cells since the dental plaque and calculus represent sites for the oral bacteria which produce the chronic bacterial infection, the chronic infection is usually lead to the chronic inflammatory process that is often associated with the human carcinogenesis and the formation of clastogenic and anuploid genetic damage in the oral epithelial cells [8]. So these indices have a prominent effect on the oral epithelial cells by increasing the rate of micronuclei. The micronuclei expression in the non-smokers resulted from the effect of the plaque and calculus indices in addition to the effect of the environmental pollutants and the passive smoking or the spicy and hot food.

In the heavy smokers' group and according to the results statistically analyzed especially on the buccal mucosa, there was a strong effect of the amalgam restorations in relation to the smoking on the micronuclei expression in the oral epithelial cells which could be attributed to that the heavy smoking will inhibit the growth of the oral bacteria and enhance the growth of tar resistant bacteria on the oral epithelial cells that have a carcinogenic effect by increase the micronuclei expression. Additional to the path of the poisonous effect of smoking on the buccal mucosa, the unfavorable effect of the amalgam restorations on the oral epithelial cells due to the direct contact of the amalgam restorations on the oral epithelial cells and the metallic ions released from these restorations. The biological interaction of the restorations with the oral epithelial cells is related to the toxic and allergic reactions and the increase in the bacterial adherence which can lead to the inflammatory effects [9].

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Table1: Micronuclei expression in the study sample

The stain / Groups	Pap stain	Giemsa stain	The total of the micronuclei
Non-smokers	52	7	59
Light smokers	74	18	92
Heavy smokers	380	122	502

Table 2: The mean and the ±SD of the micronuclei expression in Pap stain:

sites / Groups	Palate			Gingiva			Buccal mucosa			Floor of mouth		
	No.	mean	±SD	NO.	mean	±SD	No.	mean	±SD	No.	mean	±SD
Non-smokers	7	0.28	1.00	7	0.28	0	9	0.36	0	25	1.16	0.37
Light smokers	11	0.44	1.00	9	0.36	0	18	0.76	0.23	25	1.44	0.50
Heavy smokers	25	2.67	2.76	24	3.16	1.62	24	2.65	1.20	25	6.76	2.42

Table 3: The mean and the ±SD of the micronuclei expression in Giemsa stain:

Floor of mouth			Buccal mucosa			Gingiva			Palate			Sites / Groups
±SD	mean	No.	±SD	mean	No.	±SD	mean	No.	±SD	mean	No.	
0	0.24	6	_	0	0	_	0	0	_	0.04	1	**Non-smokers**
0	0.65	14	_	0.04	1	0	0.08	2	_	0	0	**Light smokers**
0.17	2.16	25	0.42	1.12	22	0.62	1	17	0.36	0.64	14	**Heavy smokers**

Table4: The statistical difference of the micronuclei expression according to the oral sites.

Giemsa stain			Pap stain			Stain / sites
P-value	df	F-test	P-value	df	F-test	
0.71 NS	1	0.14	0.0005	2	15.98	**palate**
0.31 NS	1	1.08	0.0005	2	15.30	**Gingiva**
0.61 NS	1	0.26	0.0005	2	22.25	**Buccal mucosa**
0.0005	2	18.08	0.0005	2	119.41	**Floor of mouth**

P 0.005is highly significant, NS means non- significant.

Table 5: The multiple linear regression of the micronuclei expression in the non-smokers' group.

Factors	Beta	t-test	P-value
Pl. I.	0.08	0.35	0.73
Gi. I.	-0.39	-1.57	0.15
Cal. I.	0.72	3.35	0.007
Amalgam	-0.04	-2.14	0.83
Composite	0.21	0.92	0.37

Table 6: the multiple linear regression of the micronuclei expression in the smokers' group.

Factors	Beta	t-test	P-value
Pl. I.	0.12	0.50	0.62
Gi. I.	0.11	0.50	0.62
Cal. I.	0.15	0.71	0.49
Amalgam	0.73	3.43	0.006
Composite	0.14	0.58	0.57