Genetic Damage in Exfoliated Cells of the Uterine Cervix

Association and Interaction Between Cigarette Smoking and Progression to Malignant Transformation?

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OBJECTIVE: To determine, through the micronucleus (MN) test, the cytogenetic effects of cigarette smoking on exfoliated cells from the uterine cervix in women with normal smears and women with inflammatory atypia, squamous intraepithelial lesion (SIL) (cervical intraepithelial neoplasia [CIN] 1–3) and cervical cancer.

STUDY DESIGN: The study group consisted of 200 women divided into three subgroups: group 1 (n = 116), women periodically undergoing cervical cytology and residents of Salvador-Bahia; group II (n = 57), women residing in São Paulo and previously selected because of a possible cytopathologic test positive for such conditions as human papillomavirus infections or malignant or premalignant cervical lesions (CIN 1–3); group III (n = 27), inmates of the Tatuapé Penal Institution, São

The MN test can be used, along with the cervical cytologic smear, to follow low grade lesions in women smokers.

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Paulo. All the women underwent cytologic and colposcopic examination, and biopsies were performed on 68 of them.

RESULTS: Considering the sample as a whole and using the χ^2 test for rare events, the number of MNs in smokers was significantly greater than in nonsmokers. It was also greater in women with larger exposure to smoking. The occurrence of MN was significantly lower in women with normal smears (smokers or nonsmokers) than in those showing any kind of pathologic alterations. In nonsmokers the occurrence of MN was similar between those with inflammatory atypia (IA) or low grade (L) SIL (CIN 1) and significantly higher in women with more severe lesions or high grade (H) SIL (CIN 2 or 3). Smokers with LSIL (CIN 1) showed a higher number of MNs than nonsmokers with a comparable diagnosis and smokers with IA. No differences were observed when compared with smokers with HSIL (CIN 2 and 3). MN occurrence was not associated with other risk factors for SIL or cancer development, such as age at first coitus, number of sexual partners, multiparity and use of hormonal contraceptives.

CONCLUSION: These results suggest that the mutagenic effect of cigarette smoking occurs in cervical cells and that the progression of SIL is associated with increased frequency of chromosomal damage. Moreover, the data suggest that cigarette smoking introduces an additional risk to the progression of low grade LSIL (CIN 1). MN testing would be helpful in monitoring smokers with this kind of lesion. (Acta Cytol 1998;42:639–649)

Keywords: micronucleus tests, cervix neoplasms, smoking, passive smoking, chromosome aberrations.

The epidemiology of squamous carcinoma of the uterine cervix and its precursor, squamous intraepithelial lesion (cervical intraepithelial neoplasia [CIN] grades 1–3), indicates that they are associated with several factors, most of them related to sexual behavior.[5,44] It is now widely accepted that the human papillomavirus (HPV), particularly types HPV 16 and 18, are the most important determinants of these lesions and that therefore the association with sexual factors, such as the number of sexual partners and early age at first episode of sexual intercourse, is related to a higher probability of acquiring these viruses.[1,34,40] However, several reports have consistently shown that cigarette smoking increases the risk of developing SIL and cervical cancer,[8,12,28,35,43] although other reports suggest that this association is a consequence of the fact that cigarette smoking is associated with sexual behavior.[7] Mutations in suppressor tumor genes have also been observed in a low percentage of HPV-negative tumors,[11,26,39] and both structural and numerical chromosomal aberrations have been observed in a nonrandom pattern of occurrence.[2,27,42,45,46]

The mutagenic and carcinogenic effects of several components of tobacco are well known, and their association with cancer of organs directly exposed to inhaled smoke is well established. The mechanisms of the cigarette association with SIL and cervical cancer have been discussed. It has been proposed that cigarette smoking decreases the number of Langerhans cells in the cervical epithelium, with a consequent decrease in the efficiency of immunologic defense, favoring HPV infection,[3] and it is known that some kinds of these viruses are effective at producing genetic instability.[13,20] A direct mutagenic effect on cervical mucus in smokers was described[24] but not corroborated in another, similar study.[41]

Cancer of the cervix is considered the final stage of a long and continuous process that begins with abnormalities involving cells from the upper layers of the cervical epithelium (low grade [L] SIL) (CIN 1) that progress in the direction of the innermost layers (HSIL) (CIN 2 and 3) and culminating in a process of invasion of the subjacent conjunctive tissue. The majority of lesions diagnosed as LSIL (CIN 1) spontaneously regress to normal epithelium, and only some percentage of them evolve to become invasive. The identification of lesions with potential for progression is of crucial importance. It has been proposed that lesions with this potential have aneuploid DNA content or are related to infection with oncogenic types of HPV.[4,18,38] However, today there is no available method of predicting the progression of these lesions.

In this study, the frequencies of micronuclei (MNs) were analyzed in exfoliated cells from the cervix of normal women and in women with different kinds of abnormal cervical conditions: inflammatory process, SIL and cervical cancer. MN frequency was utilized as a chromosomal aberration index to evaluate the mutagenic effects of cigarette smoking and their correlation with progression of precursor lesions. MNs are formed by chromosomal fragments or whole chromosomes that fail to be included in the nuclei during the process of cell division, resting in

the cytoplasm of interphasic cells as structures with a constitution and appearance similar to those of the nuclei.[22]

Materials and Methods

Subjects

One hundred sixteen women from Salvador (group I), who regularly underwent cervical cytology; 57 women from São Paulo examined at a cancer prevention service (Fundação Oncocentro de São Paulo) and who were previously selected because of a cervical cytology result suggestive of SIL or cancer (group II); and 27 inmates from a prison in São Paulo (group III) constituted the sample. They underwent colposcopic, colpocytopathologic and, when indicated, histopathologic examination, besides MN analysis. Consent was obtained from all women, and confidentiality was ensured. The women answered a questionnaire asking about sexual behavior, gynecologic/obstetric history, smoking and hygiene habits, and exposure to known genotoxic agents.

MN Tests

Cytologic Preparation. The material was processed according to Stich et al,[47] with modifications suggested by Gattás et al[19]: an Ayre spatula, broken in half, containing the material collected from the ectocervix was submerged in 8 mL of physiologic solution (NaCl 0.9%) and immediately transported to the laboratory for processing. The material was gently detached from the spatula using a Pasteur pipette and then subjected to 1,200 rpm of centrifugation for five minutes. The pellet was suspended in a 3/1 solution of methanol/acetic acid and centrifuged twice at the same speed for the same amount of time. Finally, the pellet was again suspended in 1 mL of methanol/acetic acid solution and dropped on clean, moist, cold slides. The slides were stained 24 hours later as per the Feulgen and Rossenbeck[17] method and fast green (1% in ethanol).

MN Analysis. The MN analysis was performed on coded slides following the criteria described by Sarto et al[37] and Tolbert et al[49]: structures were considered MNs when distinctly separated from the nucleus and with a clear contour, with a configuration and staining similar to those observed in the nuclei (Figure 1). All the cells presenting degenerative phenomena, such as karyolysis, karyorrhexis and pyknosis were excluded from the analysis.

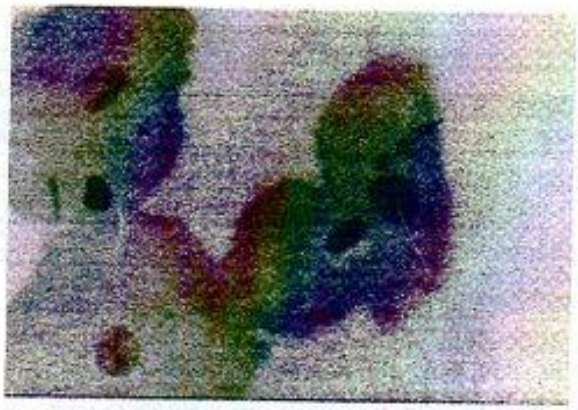

Figure 1 MN in exfoliated cervical cells (× 275).

Also excluded were cells with binucleation, nuclear buds and broken eggs.[49]

Statistical Analysis

The χ^2 test was used for tables of association to compare the three groups. Quantitative variables were evaluated using one-way ANOVA once normality was accepted. When indicated, Scheffe's test and Least Significant Difference were employed to evaluate differences between groups. The Kruskal-Wallis test and Mann-Whitney test were used when normality was not accepted.

MN data were analyzed using the conditional test for comparing proportions in situations in which events are rare.[6] This is an alternative test of significance to the χ^2 test, similar to Fisher's exact test[30] and appropriate for evaluating cytogenetic events when a great number of cells are required to detect the occurrence of chromosomal aberrations.

A sexual index (SI) was established to evaluate sexual behavior. This index considers both number of sexual partners and age at first episode of sexual intercourse as well as the time that has passed between age at first coitus and age at the moment of this investigation. It is expressed by the formula

$$SI = (t/p^2) \times \sqrt{(T-t)},$$

in which T = age, t = age at first sexual intercourse, and p = number of sexual partners.

Results

Sample Characteristics

Age. For the total women (200), mean age ± SE was 32.41 ± 0.71; for groups I, II and III it was

Table I *Data Related to the SI*

Group	No. ≤M	No. >M	$\bar{X} \pm SE$	$F_{2,197}$	Fisher least significant difference	χ^2	χ^2 Partitions
I	46	70	850.29 ± 84.05		I × II = 245.70*		I × II = 5.1245, *df* = 1, *P* = .024
II	33	24	571.30 ± 78.95	6.9090	I × III = 324.57*	14.7198	I × III = 12.7830, *df* = 1, *P* < .0005
III	21	06	285.56 ± 62.27	*P* = .0013	II × III = 354.87	*df* = 2	II × III = 3.1549, *df* = 1, *P* = .076
I+II+III	100	100	694.31 ± 56.06				

M = median = 403.50.
*Significant at 95%; *P* = .0006.

31.84 ± 0.97, 34.02 ± 1.37 and 31.44 ± 1.37, respectively. ANOVA did not show differences: $F_{2,197} = 1.0434$, $P = .3542$.

Sexual Behavior. The differences between groups relative to sexual behavior were evaluated with the SI. Significant differences between group I in relation to groups II and III were shown by ANOVA/Fisher least significant difference and χ^2 test, with SI values previously arranged by the median value (Table I).

Reproductive Variables. The obstetric history of the three groups was evaluated by considering nulliparas, women with one to three normal deliveries and no abortion or cesarean section, women with one to three deliveries and a history of abortion or cesarean section and women with more than three deliveries with or without a history of abortion or cesarean section. χ^2 was 5.9657 ($df = 6$): showing that there were no differences between groups ($P = .4270$). Data related to reproductive factors are presented in Table II.

Smoking Habits. Women smoking for at least one year, three or more cigarettes per day, and inhaling the smoke were considered smokers. Nonsmokers were considered women who had never smoked and those who had quit the habit five or more years earlier.[32,48] Smoking habits were analyzed using the χ^2 test. The results show that groups I and II were similar in terms of the number of smokers but significantly different from group III (Table III).

The level of exposure to cigarette smoking in the three groups was analyzed considering the maximum number of cigarettes per day, in a range of

Table II *Obstetric History*

Group	Nulliparas	1–3 ND, 0 A, 0 CS	1–3 ND, CS &/or A > 0	> 3 ND, with or without CS &/or A	χ^2
I	24	40	28	16	
II	10	15	14	11	5.9657
III	05	04	10	06	*df* = 6
I+II+III	39	59	52	33	*P* = .427

ND = normal delivery, A = abortion, CS = cesarean section.

Table III *Occurrence of Smokers and Nonsmokers in the Groups*

	Smokers		Nonsmokers				
Group	No.	%	No.	%	No.	χ^2 (I × II × III)	χ^2 (I+II × III)
I	40	36.0	71	64.0	111	26.8591	26.8301
II	18	34.6	34	65.4	52	*df* = 2	*df* = 1
III	24	88.9	03	11.1	27	*P* < .0005	*P* < .0005
I+II+III	82	43.2	108	56.8	190		

Table IV *Level of Exposure to Cigarette Smoke*

Group	Median	Range	Kruskal-Wallis	Mann-Whitney
I	100.00	05–450	$\chi^2 = 10.7845$	I × II ($Z = -1.4971$, $P = .1344$)
II	180.00	15–750	$df = 2$	I × III ($Z = -3.3028$, $P = .001$)
III	230.00	50–600	$P = .0046$	II × III ($Z = -1.1320$, $P = .2576$)

Table V *Cytopathologic and/or Histopathologic Diagnosis of Women Analyzed*

Group	NS	IA	KA	LSIL (CIN 1)	LSIL (CIN 1)+HPV	HSIL (CIN 2)+HPV	HSIL (CIN 3)	HSIL (CIN 3)+HPV	CA	Total
I	44	71	—	—	—	—	—	01	—	116
II	—	18	05	03	19	01	03	04	04	57
III	01	19	—	02	—	03	—	02	—	27
I+II+III	45	108	05	05	19	04	03	07	04	200

NS = normal smear, IA = inflammatory atypia, KA = koilocytic atypia, CA = cancer.

five units multiplied by duration of the habit (in years). Kruskal-Wallis/Mann Whitney test showed that women from group III were more heavily exposed than the women from groups I and II (Table IV).

Diagnostic Characterization of the Sample. Diagnoses suggestive of HPV, SIL or cervical cancer were confirmed by colposcopically guided biopsies. Sixty-eight biopsies were performed, one in the group I and 49 and 18 in groups II and III, respectively (Tables V and VI).

MN Analysis

The data related to MN analysis are presented in Table VII. The median frequencies of MN (‰) in the three groups were compared using ANOVA, and significant differences were found: $F_{2,197} = 10.0156$, $P = .0001$. The Sheffe test showed that group I was significantly different from groups II and III.

MN occurrence was also evaluated by the χ^2 test, with data previously arranged by the median value. To perform this analysis a new variable was created: 0, meaning data below the median value, and 1, equal to or above the median value. Significant differences were again shown: $\chi^2 = 14.8103$, $df = 2$, $P = .0006$. χ^2 Partitions again point to differences between group I in relation to groups II and III (Table VIII).

MN occurrence in the total sample and in the three groups in relation to age, sexual index, obstetric history (parity as presented in Table II) and hormonal contraceptive use were analyzed using the conditional test for rare events. No significant association was observed (data not shown). However, MN occurrence was higher in smokers than in nonsmokers when all the women were analyzed together. Considering each group separately, a similar result was observed in group II, but no significant difference was observed in group I. MN occurrence

Table VI *Comparative Analysis of the Distribution of Women with Normal Smears or Inflammatory or Koilocytic Atypia and Women with Different Grades of SIL*

	NS+IA+KA		LSIL + HSIL (CIN 1 + 2 + 3)			
Group	No.	%	No.	%	χ^2	χ^2 Partitions and Fisher's exact test
I	115	99	01	01	73.1800	I × II = 75.4640, $df = 1$, $P < .0005$
II	23	43	30	57	$df = 2$	II × III = 6.7718, $df = 1$, $P = .009$
III	20	74	07	26	$P < .0005$	I × III = $P < .001$
I+II+III	158	81	38	19		

For an explanation of abbreviations, see Table V.

Table VII *MN Analysis*

Group	No. of cells	Range	No. of MN observed	$\bar{X}$ (‰) ± SE	$F_{2,197}$
I	367,675	1,005–4,052	124	0.36 ± 0.04[a]	10.0156
II	159,852	1,283–3,804	102	0.72 ± 0.11[b]	P=.0001
III	82,942	1,327–3,597	66	0.83 ± 0.13[c]	
I+II+III	609,469	1,005–4,052	292	0.53 ± 0.05	

[a]Significantly different from [b] and [c].

Table VIII *MN Analysis*

	MN (M≤0.32)		MN (M>0.32)			
Group	No.	%	No.	%	χ^2	χ^2 Partitions
I	72	62.1	44	37.9	14.8103	I × II = 11.1744, *df* = 1, *P* = .001
II	20	35.1	37	64.9	*df* = 2	I × III = 7.3641, *df* = 1, *P* = .007
III	09	33.3	18	66.7	*P* = .0006	II × III = 0.0249, *df* = 1, *P* = .875
I+II+III	101	50.5	99	49.5		

M = median.

was higher in nonsmokers in group III (Table IX).

The effect of quantitative exposure to cigarette smoke was evaluated in two ways: considering the maximum number of cigarettes smoked daily and considering the exposure load (maximum number of cigarettes smoked daily multiplied by duration of the habit [in years]). In both situations, statistical analysis was performed using the χ^2 test, with the whole sample classified into three categories: nonsmokers and smokers with values above and below the median value, respectively, calculated for the two variables considered. Tables X and XI present the results obtained.

The analysis of MN occurrence in the total sample considering the diagnostic class pointed to significant differences: $\chi^2 = 76.9561$, $df = 4$, $P < .0005$. The χ^2 partition showed that MN occurrence was: (1) significantly lower in women with normal smears than in women presenting any single kind of lesion, (2) significantly lower in women showing inflammatory atypia, koilocytic atypia or LSIL (CIN 1) than that observed in women with HSIL (CIN 2 and 3) or cancer but (3) not significantly different between women with LSIL (CIN 1) when compared to those with koilocytic or inflammatory atypia and also (4) not significantly different between women with HSIL (CIN 2 or 3) in relation to those with cancer. Table XII presents the results observed.

MN occurrence in women with CIN related to HPV infection did not show differences: $\chi^2 = .046$, $df = 1$, $P > .80$ (Table XIII).

Considering the smoking habits and diagnostic class in the total sample, differences in MN occurrence were observed in both smokers and nonsmokers. The data are presented in Table XIV.

No differences in MN occurrence between smokers and nonsmokers with normal smears were ob-

Table IX *MN Occurrence in Smokers (s) and Nonsmokers (n/s)*

Group	No. s	No. n/s	MN s	MN n/s	#Cel s	#Cel n/s	MN (exp) s	MN (exp) n/s	χ^2 (*df* = 1)
I	40	71	43	78	121,795	230,378	41.8465	79.1535	0.0486 (*P* > .70)
II	18	34	47	49	54,004	90,301	35.9266	60.0734	5.4543 (*P* < .025)
II	24	03	52	14	71,917	10,025	57.9254	8.0746	4.9543 (*P* < .05)
I+II+III	82	108	142	141	247,716	330,704	121.2000	161.8000	6.2445 (*P* < .025)

#Cel = total cells.
exp = Expected.

Table X *MN Occurrence in Relation to the Maximum Number of Cigarettes Smoked Daily*

Maximum no. of cigarettes/d	MN (observed)	Total cells	MN (exp)	χ^2	χ^2 Partitions ($df = 1$)
Nonsmokers (a)	141	330,704	161.8015	6.3084	(a) × (b) = 3.6360 ($P > .05$)
No. of cigarettes ≤ M (b)	71	126,285	61.7867	$df = 2$	(a) × (c) = 4.7485 ($P < .05$)
No. of cigarettes > M (c)	71	121,431	59.4118	$P < .05$	(b) × (c) = 0.0545 ($P > .80$)
Total	283	578,420	283.0000		

M = median = 10.
exp = Expected.

Table XI *MN Occurrence in Relation to Exposure*

Exposure	MN (observed)	Total cells	MN (exp)	χ^2	χ^2 Partitions ($df = 1$)
Nonsmokers (a)	141	330,704	161.8015	6.4437	(a) × (b) = 3.3454, $P > .05$
Exposure ≤ M (b)	73	131,624	64.3989	$df = 2$	(a) × (c) = 5.1595, $P < .025$
Exposure > M (c)	69	116,092	56.7996	$P < .05$	(b) × (c) = 0.1700, $P > .60$
Total	283	578,420	283.0000		

M = median = 1.
exp = Expected.

served. There were no significant differences between smokers and nonsmokers with inflammatory atypia. Cells of smokers with LSIL (CIN 1), however, had a significantly nigher number of MNs than in nonsmokers. No differences were observed when comparing cytologic preparations from smokers and nonsmokers with a large number of precursor lesions (Table XV).

The analysis of MN occurrence in smokers with normal smears showed results similar to those observed without considering this habit. Smokers with LSIL (CIN 1), however, had MN numbers significantly higher than observed for smokers with inflammatory atypia and similar to that for women with high grade lesions (Table XVI).

In nonsmokers, analysis of MN occurrence showed results similar to those recorded without considering smoking habits for all diagnostic classes considered (Table XVII).

Discussion

MN occurrence was evaluated in three groups in relation to several factors that have been observed in association with SIL and cervical cancer. Except for multiparity, women from group I were less exposed to all analyzed variables when compared to women from groups II and III, and cervical lesions were most common in the last two groups. This was not surprising since women from group I regularly underwent Papanicolaou examination, in contrast to women from groups II and III. Furthermore, women from group II constituted a sample previously selected by colposcopic and cytopathologic analysis suggesting cervical abnormalities. MN oc-

Table XII *MN Occurrence in Normal Women and Women with Different Diseases*

Diagnosis	Total cells	MN (observed)	MN (exp)	χ^2	χ^2 Partitions ($df = 1$)
Normal smear (a)	145,388	27	69.656		(b) × (c) = 1.1531 ($P > .20$)
IA + KA (b)	345,235	166	165.404	76.9561	(b+c) × (a) = 25.0411 ($P < .0005$)
LSIL (CIN 1) (c)	65,171	38	31.224	$df = 4$	(b+c) × (d) = 24.8378 ($P < .0005$)
HSIL (CIN 2+3) (d)	43,086	47	20.643	$P < .0005$	(d) × (e) = 0.4000 ($P > .50$)
Cancer (e)	10,589	14	5.073		(d+e) × (a) = 80.1601 ($P < .0005$)
Total	609,469	292	292.000		

IA = inflammatory atypia, KA = koilocytic atypia, exp = expected.

Table XIII *MN Occurrence in Women with CIN Related to HPV Infection*

Diagnosis	N	No. of cells	MN (observed)	MN (exp)	χ^2
CIN + HPV	30	86,161	65	65.8267	0.046
CIN without HPV	08	25,096	20	19.1733	*df* = 1
Total	38	111,257	85	85.0000	*P* > .80

No. of cells = total cells.
exp = Expected.

Table XIV *MN Occurrence in Smokers and Nonsmokers in Relation to Diagnostic Class*

Diagnosis	No. of cells (smokers)	MN (observed) (smokers)	MN (exp) (smokers)	No. of cells (nonsmokers)	MN (observed) (nonsmokers)	MN (exp) (nonsmokers)	χ^2 Smokers	χ^2 Nonsmokers
Normal smear	47,111	09	27.0058	91,938	18	39.1990	55.5955	41.1099
IA + KA	152,316	80	87.3132	177,755	81	75.7882	*df* = 4	*df* = 4
LSIL (CIN 1)	15,142	15	8.6799	40,483	19	17.2605	*P* < .0005	*P* < .0005
HSIL (CIN 2+3)	30,119	29	17.2653	12,967	18	5.5286		
Cancer	3,028	09	1.7358	7,561	05	3.2237		
Total	247,716	142	142.0000	330,704	141	141.0000		

No. of cells = total cells, exp = expected, IA = inflammatory atypia, KA = koilocytic atypia.

currence in women in group I was also significantly lower than that observed for the other two groups. It suggests, *a priori*, that different risk factors could be associated with cytogenetic damage in these groups of women or that the differences observed were a consequence of the differential distribution of pathology between the groups.

MN occurrence was not associated with aging. Higher frequencies of MN have been recorded for women past menopause when compared to those undergoing it.[10] An increase in MN frequency associated with aging has been also observed for lymphocytes[16] but not in exfoliated cells from the buccal mucosa.[37]

The association of sexual behavior with SIL or cervical cancer reflects the probability of acquiring oncogenic HPV and other infectious agents. These infections increase cellular proliferation, favoring the occurrence of mitotic errors. The greater occurrence of MN in women with inflammatory atypia than that observed in women without cervical abnormalities observed in this study supports this idea. It has been suggested that the young epithelium is more susceptible to the action of carcinogenic and infectious agents with oncogenic potential[14,23]; that could be associated with a high occurrence of cytogenetic damage. The MN test, however, reflects only recent chromosomal damage, and in the majority of the women analyzed, a long time passed between the first episode of sexual intercourse and MN analysis.

We did not observe any association between

Table XV *MN Occurrence, by Diagnostic Class, in Relation to Smoking Habits*

Diagnosis	No. of cells (smokers)	MN (observed) (smokers)	MN (exp) (smokers)	No. of cells (nonsmokers)	MN (observed) (nonsmokers)	MN (exp) (nonsmokers)	χ^2
Normal smear	47,111	09	9.1478	91,938	18	17.8522	0.0036, *df* = 1, *P* > .95
IA+KA	152,316	80	74.2958	177,755	81	86.7042	0.8132, *df* = 1, *P* > .30
LSIL (CIN 1)	15,142	15	9.2553	40,483	19	24.7447	4.8994, *df* = 1, *P* < .05
HSIL (CIN 2+3)	30,119	29	32.8551	12,967	18	14.1449	1.5030, *df* = 1, *P* > .20
Cancer	3,028	09	4.0034	7,561	05	9.9966	8.7336, *df* = 1, *P* < .005

No. of cells = total cells, exp = expected, IA = inflammatory atypia, KA = koilocytic atypia.

Table XVI *MN Occurrence in Smokers, by Diagnostic Class*

Diagnosis	Total cells	MN	χ^2 Partitions
Normal smear (a)	47,111	09	(a) × (b) = 9.0043, *df* = 1, *P* < .005
IA + KA (b)	152,316	80	(b) × (c) = 5.2583, *df* = 1, *P* < .025
LSIL (CIN 1) (c)	15,142	15	(c) × (d) = 0.0080, *df* = 1, *P* > .90
HSIL (CIN 2+3) (d)	30,119	29	(b) × (c+d) = 11.1044, *df* = 1, *P* < .005
Cancer (e)	3,028	09	(a) × (c+d) = 24.5462, *df* = 1, *P* < .0005
Total	247,716	142	χ^2 = 55.5955, *df* = 4, *P* < .0005

IA = inflammatory atypia, KA = koilocytic atypia.

MNs and multiparity. It has been shown that the metaplastic process, which is characterized by a high rate of cellular proliferation, accelerates during pregnancy,[33] leading to a high probability of genetic damage. The absence of any association with age at first episode of intercourse might be due to the fact that MN reflects only recent injuries.

The higher MN occurrence observed in women smokers than in nonsmokers in group II, and also in the whole sample, suggests that genotoxic effects of cigarette compounds are observed in cells from the cervix. This effect was also observed in other cell types.[15,25,36,37,50,51] The observation that the heaviest smokers showed a higher number of MNs than nonsmokers supports this suggestion and could indicate a dose-dependent effect. This suggestion is supported by the observation that MN occurrence in nonsmokers and smokers of a few cigarettes per day was not different. Dose-dependent effects are probably responsible for the similar occurrence of MNs in smokers and nonsmokers from group I since they were light smokers. Although statistical analysis did not point to differences in the number of cigarettes smoked daily and to exposure between women from group II in relation to the two other groups, probably because of a statistical error of type II, the absolute values obtained for these variables were higher in women from this group in relation to women from group I. The higher number of MNs in nonsmokers from group III could be due to several factors. One, nonsmokers from this group were exposed passively since they lived with heavy smokers. It has been shown that the levels of nicotine in cervical mucus from nonsmokers passively exposed to smoke are greater than those observed in nonsmokers without this kind of exposure.[29] Furthermore, it has also been shown that sidestream smoke contains more nitrosamines than inhaled smoke.[9] Two, one of the nonsmokers was infected with herpes simplex virus, which causes genetic instability leading to chromosomal damage.[21] Last, there were only three nonsmokers in this group, and so the results could reflect sampling error.

Our findings on MN occurrence in women with cervical cancer are not informative since only a few cases were included in this sample. The association between cytogenetic damage and HPV infection in women with SIL demands further study. Viral typing was not done, and it is possible that genetic instability from HPV infection is a function of the type of virus.

The higher number of MNs in women with inflammatory atypia, when compared to that observed in women with normal smears, suggests that high rates of cell proliferation are efficient at producing genetic damage. The equal frequency of MNs among women with inflammatory atypia and LSIL (CIN 1) suggests that the initial dysplastic changes are not accompanied by a higher rate of

Table XVII *MN Occurrence in Nonsmokers, by Diagnostic Class*

Diagnosis	Total cells	MN	χ^2 Partitions
Normal smear (a)	91,938	18	(a) × (b) = 11.1505, *df* = 1, *P* < .001
IA + KA (b)	177,755	81	(b) × (c) = 0.0134, *df* = 1, *P* > .90
LSIL (CIN 1) (c)	40,483	19	(a) × (d) = 47.0800, *df* = 1, *P* < .0005
HSIL (CIN 2+3) (d)	12,967	18	(d) × (b+c) = 20.7385, *df* = 1, *P* < .0005
Cancer (e)	7,561	05	(a) × (c+d) = 22.0209, *df* = 1, *P* < .0005
Total	330,704	141	χ^2 = 41.1099, *df* = 4, *P* < .0005

IA = inflammatory atypia, KA = koilocytic atypia.

chromosomal damage. The higher number of MNs observed in women with HSIL (CIN 2 and 3), when compared with that observed in women with LSIL (CIN 1), suggests that the progression to malignant transformation involves an increase in the frequency of chromosomal damage.

It is known that a considerable number of precursor lesions spontaneously regress and that the potential for progression is greater in high grade lesions than in low grade lesions. The identification of lesions with potential for progression is crucial for determining therapy. It has been proposed[31] that 50–90% of lesions classified as LSIL (CIN 1) and that show regression are related to nononcogenic HPV. Such lesions are considered different diseases from HSIL (CIN 2 and 3); they do not represent cancer precursors, although a low percentage of them progress to HSIL and cancer. This suggests that the disease progression could be associated with the kind of virus. Therefore, it is possible that in the absence of the virus, genetic damage could be associated with evolving behavior in LSIL (CIN 1). It is possible that the progression of lesions is associated with a high proportion of chromosomal damage, possibly as a consequence of some advantage acquired from karyotypic changes. The higher proportion of MNs observed in high grade lesions would reflect previous events. The presence of a high number of MNs in an LSIL (CIN 1) lesion could be considered a marker of a higher probability of disease progression.

Cigarette smoking introduces an additional risk to chromosomal damage. Smokers with LSIL (CIN 1) could have a higher risk of progression to high grade lesions as a consequence of a greater proportion of genetic damage. Thus, the MN test can be used, along with the cervical cytologic smear, to follow low grade lesions in women smokers.

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