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A cytogenetic study on workers in leather tanning industry

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Abstract:

Peripheral blood samples were obtained from thirty three leather tannery workers directly involved in the tanning process in the tanning factory at Al- Zaafarniya / Baghdad , one of the factories of state company for leather tanning industries. In addition peripheral blood from twenty healthy control individuals (not known to be exposed to chemicals or other potentially genotoxic substances) were used as control group.

The micronucleus (MN) assay was performed using the cytochalasin B technique. We observed a highly significant increase in the mean frequency of lymphocyte micronuclei (MN) and nucleoplasmic bridges (NPBs) , a significant increase in the mean frequency of nuclear buds (NBUDs) and a highly significant decrease in the mean frequency of nuclear division index (NDI) of the tannery workers group in comparison with the control group.

The results of the present study also indicate the influence of service duration of tannery workers in lymphocyte MN , NPB, NBUD and NDI frequencies . The age of tannery workers also effect in lymphocyte micronuclei and nuclear division index frequency.

The conclusion from our study that the tannery factory workers studied have experienced genotoxic exposure, which is manifested as an increase in the frequency of lymphocyte MN, NPB, NBUD and a decrease in NDI this work shows a clear genotoxic effect. Our study recommends providing all occupational safety requirements for workers in the factory and commitment of workers apply them.

Key word: *Micronucleus assay , leather tanning , cytochalasin B technique, genotoxicity.*

Introduction:

Genotoxicity biomarkers considered as tools for detecting human genotoxic exposure and effects dealing with occupational exposure to chemical carcinogens (1). Micronucleus (MN) assay is one of the genotoxic biomarkers that have been used as an indicator of chromosome damage (2).

Micronucleus (MN) assay in peripheral blood lymphocytes is well established as a standard method for human biomonitoring studies to detect cytogenetic effects in populations exposed occupationally and environmentally to genotoxic chemicals which can be used to identify risk from exposure to environmental and occupational pollutants well before the clinical onset of disease (3).

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The assay include scoring MN as a measure of chromosome breakage and chromosome loss, detection of nucleoplasmatic bridges (NPB), a biomarker of DNA misrepair and/or telomere end-fusions, and nuclear buds (NBUD), a biomarker of elimination of amplified DNA and/or DNA repair complexes. The nuclear division index (NDI) is a bio¬marker that measure the proliferative status of the viable cell (4).

Leather tanning industry involves many procedures with different chemical exposures, which can be harmful for the health tannery workers and particularly be carcinogenic (5). Tannery workers exposed to a variety of chemical agents, some of them being well identified carcinogens (solvents, enzymes natural and synthetic oil, sulphuric acid, formic acid, alkaline chromium sulfate , organic and inorganic dyes ,phosphoric ether, sulphate, carbonate, melanin, etc.) (5).

Many chemicals agents are stable and permanent environmental contaminants and have the potential to cause genetic

alterations in the target tissues of humans exposed to it (6), therefore, the aim of the present study was to evaluate the incidence of genetic damage in workers chronically exposed to chemical hazards using micronucleus (MN) assay in peripheral lymphocytes.

Materials and Methods:

Subjects

Heparinized venous blood samples were obtained from tannery workers (n=33 , 27 male and 6 female) directly involved in the tanning process or the finishing department in the tanning factory at Al- Zaafarniya / Baghdad , one of the factories of state company for leather tanning industries. The tannery workers age range 27 to 61 years old (mean 44.375). The duration of their work range 4 to 34 years (a mean working time of 16.129 years).We also collected blood from twenty healthy control individuals (n=20 , 10 male and 10 female) not known to be exposed to chemicals or other potentially genotoxic substances.

The study groups (tannery workers group , healthy control individuals group) live in different areas of the city of Baghdad . A questionnaire on age, occupational history, marital status, health status, recent radio diagnostic exposure, drug consumption , family history of cancer , smoking and drinking habit was applied to all subjects.

Micronucleus (MN) assay

The MN assay was performed using the cytochalasin B technique as described by Fenech and Morely (7) with some minor adaptations. Briefly, 0.5 ml whole blood was added to 4.5 ml RPMI 1640 medium (US Biological ,USA) , supplemented with 20% fetal bovine serum , streptomycin–penicillin antibiotics, 0.4 ml phytohemagglutinin (PHA) and incubated at 37C□.

Cytochalasin B (Santa Cruz biotechnology Inc, USA, cat # sc-3519) was added at 44 hour of incubation at final concentration of 4.5µg per ml culture. After a total incubation time of 72 h at 37C□, cells were harvested by centrifugation, resuspended with 10 ml hypotonic solution (0.075M KCl) for 5 minutes at 37C□ followed by immediate fixation with freshly prepared cold methanol:acetic acid (6:1, v/v) . Cell suspensions were dropped onto pre-chilled slides, air dried and stained with Giemsa. Slides were coded and scored by light microscopy at 1000X magnification.

For each subject, 1000 binucleated (BN) lymphocytes with well-preserved cytoplasm were scored. Binucleated cells with micronuclei (BNMN) , nucleoplasmic bridges (NPB) and nuclear buds (NBUD) were identified according to the criteria of Fenech et al., 2003 (8).

We examined 500 lymphocytes to evaluate the nuclear division index (NDI) using the formula NDI=M1+2M2+3M+4M/N, where M1-M-4 represent cells with 1, 2, 3, or 4 nuclei, and N is the total number of viable cells scored (9).

Statistical analysis

All statistical analysis was conducted using Statistical Package for Social Sciences (SPSS) version 22. Data were given as mean ± standard error (SE), while significant differences between means were assessed by t- tests. The comparison of significant (p-value) were : significant difference ($P < 0.05$) , Highly significant ($p < 0.01$) and Non-Significant difference ($p > 0.05$) (10).

Results:

We used the MN assay to assess whether the occupational exposure of the tanning factory workers could exert some genotoxic effects. As shown in table (1), figure 1, we observed highly significant increase ($p < 0.01$) in the mean frequency of binucleated lymphocytes with micronuclei (BNMN) and highly significant decreases ($p < 0.01$) in the mean frequency nuclear division index (NDI) of the tannery workers group in comparison with the control group.

A highly significant increase ($p < 0.01$) in the mean frequency of binucleated lymphocytes with nucleoplasmic bridges (NPB) (table 1, figure 2) and a significant ($p < 0.05$) increase in the mean frequency of nuclear buds (NBUD) (table 1, figure 3).

The result of our study showed highly significant increase ($p < 0.01$) in the mean frequency of BNMN , NPB and NBUD and a highly significant decrease ($p < 0.01$) in NDI of the tannery workers group with service duration > 15 year in comparison with the tannery workers with service duration ≤ 15 year as shown in table 2.

Tannery workers with age group > 45 year showed significant increase ($p < 0.05$) in the mean frequency of BNMN and a significant decrease ($p < 0.01$) in NDI when compared with tannery workers with age group ≤ 45 year (table 3).

Furthermore the result of our study revealed no significant difference ($p > 0.05$) in the mean frequency of BNMN and NDI in in lymphocyte of tannery workers according to gender (table 4) and smoking habit (table 5). No statistical difference ($p > 0.05$) was detected in lymphocyte frequency of NPB and NBUD in tannery workers according to service duration , age , gender and smoking habit (table 2, 3,4 and 5).

Table1. Mean (± SE) frequencies of MN, NPB, NBUD and NDI in lymphocytes of studied groups.

Parameters	group		Comparison of Significance	
	Tannery workers (n=33)	Control group (n=20)	p- value	Significance
MN/1000 BN cell Mean ± SE	16.48 ± 1.31	0.99 ± 5.95	0.000	Highly significant (p<0.01)
NPB/1000 BN cell Mean ± SE	3.70±0.67	0.55±0.29	0.001	Highly significant (p<0.01)
NBUD/1000 BN cell Mean ± SE	1.06±0.26	0.20±0.12	0.061	Significant (p<0.05)
NDI Mean ± SE	1.38±0.05	1.82±0.07	0.000	Highly significant (p<0.01)

Table 2. Mean (± SE) frequencies of MN, NPB, NBUD and NDI in lymphocytes of tannery workers according to service duration.

Parameters	15 ≥ (n= 17)	15< (n=16)	p- value	Significance
MN/1000 BN cell Mean ± SE	11.82±1.27	21.44±1.59	0.000	Highly significant (p<0.01)
NPB/1000 BN cell Mean ± SE	0.88±0.30	6.69±0.85		
NBUD/1000 BN cell Mean ± SE	0.12±0.08	2.06±0.39		
NDI Mean ± SE	1.56±0.06	1.19±0.02		

Table 3. Mean (± SE) frequencies of MN, NPB, NBUD and NDI in lymphocytes of tannery workers according to age.

Parameters	Age (years)		Comparison of Significance	
	45 ≥ (n= 16)	45< (n=17)	p- value	Significance
MN/1000 BN cell Mean ± SE	13.81±1.32	19±2.07	0.04	Significant (p<0.05)
NPB/1000 BN cell Mean ± SE	2.50±0.84	4.82±0.98	0.084	Not significant (P>0.05)
NBUD/1000 BN cell Mean ± SE	0.63±0.33	1.470.37	0.10	Not significant (P>0.05)
NDI Mean ± SE	1.49±0.07	1.28±0.05	0.017	Significant (p<0.05)

Table 4. Mean (± SE) frequencies of MN, NPB, NBUD and NDI in lymphocytes of tannery workers according to gender.

Parameters	Gender		Comparison of Significance	
	Male (n= 27)	Female (n=6)	p- value	Significance
MN/1000 BN cell Mean ± SE	16.44±1.51	16.67±2.64	0.94	Not significant (P>0.05)
NPB/1000 BN cell Mean ± SE	4.26±0.77	1.17±0.60	0.075	Not significant (P>0.05)
NBUD/1000 BN cell Mean ± SE	1.26±0.30	0.17±0.17	0.1	Not significant (P>0.05)
NDI Mean ± SE	1.38±0.05	1.40±0.05	0.87	Not significant (P>0.05)

Table 5. Mean (± SE) frequencies of MN, NPB, NBUD and NDI in lymphocytes of tannery workers according to smoking habit.

Parameters	smoking status		Comparison of Significance	
	Smokers (n= 9)	Non-smokers (n=24)	p- value	Significance
MN/1000 BN cell Mean ± SE	18.67±2.67	15.67±1.50	0.31	Not significant (P>0.05)
NPB/1000 BN cell Mean ± SE	3.11±1.38	3.92±0.78	0.60	Not significant (P>0.05)
NBUD/1000 BN cell Mean ± SE	1±0.5	1.08±0.31	0.88	Not significant (P>0.05)
NDI Mean ± SE	1.37±0.09	1.39±0.05	0.84	Not significant (P>0.05)

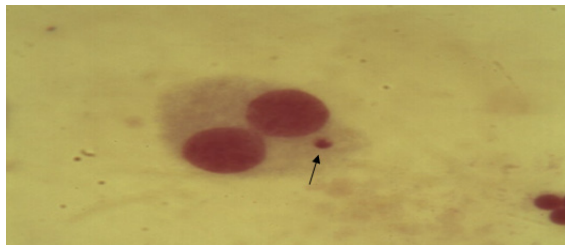

Figure 1. Binucleated lymphocyte with micronucleus , under 1000X magnification.

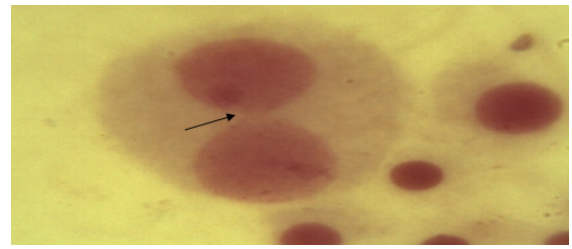

Figure 2. Binucleated lymphocyte with nucleoplasmic bridge , under 1000X magnification.

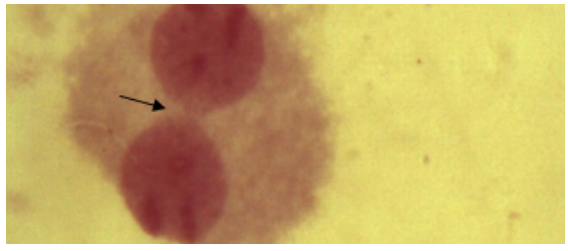

Figure 3. Binucleated lymphocyte with nuclear bud , under 1000X magnification.

Discussion:

The result of our study revealed a highly significant ($p<0.01$) increase in the mean frequency of binucleated lymphocytes MN and NPB, a significant ($p<0.01$) increase in the mean frequency of NBUD and a highly significant ($p<0.01$) decrease in the mean frequency of NDI of the tannery workers group in comparison with the control group.

Since the formation of micronuclei is related to DNA lesions resulting in acentric fragments or whole chromosomes not being included in the resulting nuclei during mitosis (11,12). NPBs provide a measure to the extent of chromosome rearrangement, NBUDs are ¬markers of gene amplification and NDI is a bio¬marker that measure the proliferative status of the viable cell frac¬tion (9). An increase in the mean frequency of in NPB and NBUD were observed in the tannery workers group in comparison with the control group suggesting chromosomal damage and a DNA instability status in this group (9,13).

A decrease in the mean frequency of NDI of the tannery workers group in comparison with the control group suggest¬ing a possible cytostatic effect induced by chemical agents involved in leather tanning industry procedures and /or a delay in the cellular division process in order to preferable repair their DNA damages (14). This result seems to indicate a higher genotoxic risk in the tannery population. However, leather processing involves a considerable number of potentially genotoxic substances which may be contributing to the cytogenetic lesion reported (15).

The results of the present study also indicate the influence of service duration in the MN ,NPB , NBUD and NDI biomarkers among tannery workers . The increases frequencies of MN , NPB , NBUD and the decrease frequencies of NDI which reflect the cumulative effect of the complex mixture of chemical agents involved in leather tanning industry procedures (16) , which had been reported previously (17-19).

The increase frequencies of MN, and the decrease frequencies of NDI were statistically significant in tannery workers with age group > 45 year when compared with tannery workers with age group ≤ 45 year. Aging characterized by instability in the organization and expression of the genetic material that reflect in different genetic end-points such as DNA breaks, mutation at different genetic loci and chromosomal damage (20). Recent studies showed that stable genetic changes accumulated with age (21-25).

No statistical significant for MN , NPB, NBUD and NDI according to gender and smoking habit of tannery workers was detected which may be attributed to the big difference in the number of male (n=27) compared to female(n=6), and smokers (n=9) compared to non –smokes (n=24) , that is why the comparison between tannery workers and control group according to gender and smoking habit cannot be ascertained .

In conclusion, our results indicate that the tannery factory workers studied have experienced genotoxic exposure, which is manifested as an increase in the frequency of MN, NPB, NBUD and a decrease in NDI this work shows a clear genotoxic effect. In this context, our positive results would reinforce the higher sensitivity of MN assay in the biomonitoring of occupationally exposed populations. Our study recommends providing all occupational safety requirements for workers in the factory and commitment of workers apply them.

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دراسة وراثية خلوية على العمال في صناعة دباغة الجلود

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الخلاصه:

تم الحصول على عينات الدم المحيطي لثلاثة وثلاثون من عمال دباغة الجلود المشاركون بشكل مباشر في عملية الدباغة في مصنع دباغة الزعفرانية / بغداد ، احد مصانع الشركة العامة للصناعات الجلدية .فضلا عن استخام الدم المحيطي من عشرون فردا كمجموعة سيطرة (لايتعرضون لمواد كيميائية او مواد سمية اخرى).

تم اجراء فحص النواة الصغرى باستخدام تقنية cytochalasin B . لوحظ وجود زيادة بفرق معنوي عالي في معدل تكرار الانوية الصغيرة و الجسور البلازمية النووية للخلايا اللمفاوية وزيادة بفرق معنوي في معدل تكرار البراعم النووية ونقصان بفرق معنوي عالي في معامل الانقسام النووي في مجموعة عمال الدباغة بالمقارنة بمجموعة السيطرة .اشارت النتائج ايضا الى تأثير مدة الخدمة على تكرار الانوية الصغيرة ، الجسور البلازمية النووية، البراعم النووية و معامل الانقسام النووي في الخلايا اللمفاوية . عمر عمال الدباغة ايضا اثر في تكرار الانوية الصغرى ومعامل الانقسام النووي .

الاستنتاج من هذه الدراسة الى ان مجموعة عمال الدباغة المدروسة تعرضوا للتسمم الوراثي والذي يتجلى في زيادة تكرار الانوية الصغرى ، الجسور البلازمية النووية، البراعم النووية و معامل الانقسام النووي في الخلايا اللمفاوية ويظهر في هذه الدراسة تاثير السمية واضح . توصي دراستنا بتوفير كافة متطلبات السلامة المهنية للعاملين في المصانع والتزام العاملين في تطبيقها.