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CYTOGENETIC BIOMONITORING OF CHEMISTRY LABORATORY WORKERS OCCUPATIONALLY EXPOSED TO TOXIC CHEMICALS

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ABSTRACT

Micronuclei (MN) in buccal cells are widely used as biological markers to estimate the genetic risk of exposure to complex mixtures of toxic chemicals. Many laboratory workers of various courses in educational and research institutes are not formally trained or not adequately educated in the use of safety protocols while handling such chemicals. They work with chemicals without use of hand gloves and safety goggles; and often do not follow safety protocols associated with its usage and disposal. Due to their ignorance and lack of awareness, they are unknowingly exposed to toxic chemicals in the form of fumes, vapours, liquids or solids. Hence, the micronuclei assay may be used as a potential cytogenetic marker of DNA damage providing an efficient and cost effective way to identify and quantify the exposure to toxic chemical substances. The assay may also be used for risk assessment and in understanding early biological effects of both occupational and environmental exposure to toxic chemicals. In our study we found that there was a significant increase in the frequency of MN in subjects exposed to occupational chemicals than the control group: MN in subjects was found to be 66 (n=50) while only 21 MN were observed in control (n=50). There was a considerable increase of 9 MN observed in alcoholic subjects (n=5) as against 5 MN seen in alcoholic control. Non-alcoholic subject population exhibited an increase of 37 MN over non-alcoholic control. The obtained number of MN in non smokers was 57 (n=50) which was higher than the 19 MN observed in the control (n=50).

KEYWORDS: Cytogenic monitoring, Micronuclei, Smoking, Alcohol consumption.

INTRODUCTION

In the last few decades, genotoxicity biomarkers have received wide attention as tools for identifying genetic aberrations due to exposure to toxic chemicals. This is particularly observed in health observation programs dealing with occupational exposures. Populations of industrial areas are largely exposed to chemicals that can cause mutations, cancer and other genetic defects (Hirvonen, 1995). Biomarkers of early biological effects like formation of micronuclei (MN) are hence very helpful in monitoring populations exposed to genotoxic agents (Hagmar *et al.*, 2004; Bonassi *et al*., 2004; Tailoli *et al*., 2007). The evaluation of MN is one of the most commonly used techniques involving the use of buccal epithelial cells and lymphocytes for the assay (Battershill *et al*., 2008). MN is a sensitive marker for detecting DNA damage, and has been used to investigate genotoxicity of a variety of chemicals. MN testing with interphase cells is more suited as a cytogenetic marker because it is not limited to metaphase and has the advantage of allowing rapid screening of a larger numbers of cells than in studies with sister chromatid exchanges or chromosomal aberrations (Ishikawa *et al*., 2003).

MN is described as a small nucleus which appears in the form of chromosome or as tiny fragments of chromosomes which are not incorporated into one of the daughter nuclei during cell division. Any deviation in normal nuclear division during anaphase stage will lead to lagging of chromosome fragment or whole chromosome, which eventually results in the formation of micronuclei. MN is most commonly found in cytoplasm of erythrocytes.

MN were formerly termed as “fragments of nuclear material” or “Howell-Jolly bodies”. Their formation results in the daughter cell lacking a part or all of a chromosome. During mitosis, dividing cells either contain chromosomes breaks lacking centromeres or whole chromosome that are unable to travel to the spindle poles (Falck *et al.*, 2002). “Laggards” or lagging chromosomes cannot move to the poles, because they are detached from the mitotic spindle (Cimini *et al.*, 2002) and tend to possess bipolar orientation. These lagging chromosome and fragments gets encapsulated by nuclear envelop to form structures similar to interphase nucleus. The lagging elements are included in the daughter cells too, but a considerable proportion is transformed into one or several secondary nuclei, which are, as a rule, much smaller than the principal nucleus and are therefore called micronuclei (MN) (Fenech, 2000). Bigger MN result from exclusion of whole chromosome following damage to the spindle apparatus of the cell (aneugenic effect), whereas smaller MN result from structural

aberrations; causing chromosomal fragments (clastogenic effect). Besides these fundamental mechanisms, some MN may have their origin in fragments derived from broken anaphase bridges (Saunders *et al.*, 2000) formed due to chromosome rearrangements such as dicentric chromatids, intermingled ring chromosomes or union of sister chromatids.

Most of the genomic toxicity study involving invasive techniques are not only very costly, but also time consuming. In this milieu, a safer, more economical and non-invasive method of obtaining exfoliated buccal cells in various disease condition and circumstances to study MN is a more viable proposition (Mukherje *et al*., 2011).

MATERIALS AND METHODS

The individuals chosen were divided into two categories. The first category (subjects) consisted of chemistry laboratory workers occupationally exposed to chemicals (n=50). The second category (control) comprised of individuals not exposed to occupational chemicals (n=50). Both subjects and control were urban citizens. The individuals were grouped as per age (Table 1). The questionnaire given to both groups had two sections: personal information and knowledge based information. Buccal epithelial sample was obtained by gently scrapping the inner part of cheek and gums with the help of smooth edged spoon. The spoon was washed in a small container using 3 ml of Tris-EDTA Buffer (pH 6.8). The sample bottles were labeled carefully. The sample was then subjected to centrifugation at 8000 rpm for 10 min. A uniform smear of the pellet was prepared on a clean grease-free slide and allowed to air dry. The cells were fixed using Cornoy's fixative (methanol and glacial acetic acid (3:1) overnight. The slides were stained with Giemsa stain for 20 min, washed with distilled water, blotted and observed under 45X objective of a compound microscope. The numbers of MN were recorded. The slides containing MN were made permanent using DPX and paraffin gel.

To avoid errors the following precautions were taken:

1) Sample bottles and slides were labeled carefully.
2) Slides were prepared in duplicates for the accuracy and reproducibility of results.
3) Slide evaluation was done by three individuals to reduce human error and the questionable cells were rechecked and reconsidered.

RESULT AND DISCUSSION

The study was done on 100 individuals out of whom 50 were subjects (34 men, 16 women) and 50 were control (24 men, 26 women). The average age was 35.7 years for men and 31.3 years for women. For subjects, the average number of years and duration of exposure to laboratory chemicals in subjects was 10.2 years and 6.6 h daily respectively. The dietary preference of individuals and those who habitually smoke and consume alcohol is given in Table 2. The descriptive chart for individuals belonging to subject and control group is as in Tables 3 and 4 respectively.

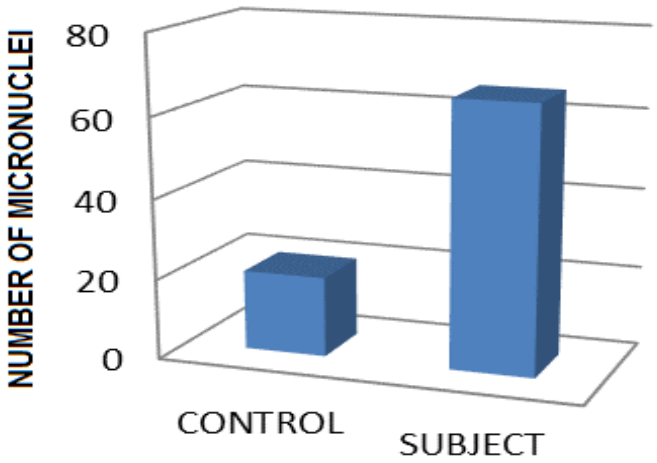

Fig 2: Number of micronuclei (MN) observed in control and subject.

Table 1: Grouping of individuals based on age.

Group	Age (years)	N	Minimum	Maximum	Mean ± SD
Group 1	20-30	13	23	29	26.00 ± 2.5
Group 2	30-40	25	30	39	35.48 ± 2.5
Group 3	40-50	9	40	49	44.56 ± 3.3
Group 4	50-above	3	51	59	54.00 ± 4.3

Table 2: Dietary preference of individuals in the subject and control groups.

Sl. No	Variables	Total Number	Subject	Control
1	**Number (n)**	100	50	50
2	**Average age (years)**		35.7	31.3
3	**Sex**			
	Male	58	34	24
	Female	42	16	26
4	**Smoking habit**			
	Non smoker	95	46	49
	Smoker	5	4	1
5	**Drinking habit**			

	Non alcoholic	89	45	44
	Alcoholic	11	5	6
6	**Dietary habits**			
	Vegetarian	37	21	16
	Non Vegetarian	63	29	34
7	**Average duration of exposure to chemicals (years)**	10.22		
8	**Average duration of exposure to chemicals on daily bases (h)**	6.6		
9	**Average number of MN per 1000 buccal cells**	1.32		0.40

Table 3: Descriptive chart for subject individuals.

CODE	AGE (years)	SEX	EXPERIENCE as lab technican (years)	Hours of exposure (h)	Diet V/NV	Alcohol consumption A/NA	Smoking habit S/NS	Micronuclei M/NM
T1	59	M	34	9	V	NA	NS	M(5)
T2	38	M	16	8	NV	NA	NS	NM
T3	48	M	23	6	V	NA	NS	NM
T4	40	M	3	6	V	NA	NS	NM
T5	33	M	12	10	NV	A	NS	NM
T6	35	M	4	2	NV	NA	NS	NM
T7	30	M	6	9	NV	NA	NS	NM
T8	24	F	5	8	NV	NA	NS	M(2)
T9	24	F	4	8	NV	NA	NS	M(2)
T10	23	M	4	6	NV	NA	NS	M(13)
T11	23	F	4	6	NV	NA	NS	NM
T12	24	F	4	3	V	NA	NS	M(1)
T13	30	F	3	8	V	NA	NS	NM
T14	33	M	14	8	NV	A	NS	NM
T15	37	M	16	9	NV	A	S	M(6)
T16	34	M	6	8	NV	NA	NS	NM
T17	42	M	20	8	V	NA	NS	M(1)
T18	37	M	18	7	NV	NA	NS	NM
T19	43	M	20	7	V	NA	NS	M(3)
T20	24	M	2	8	V	NA	NS	NM
T21	27	M	1	8	NV	NA	NS	NM
T22	37	M	2	5	NV	NA	NS	NM
T23	39	M	10	8	V	NA	NS	NM
T24	36	M	11	8	V	NA	NS	NM
T25	44	F	6	6	NV	NA	NS	NM
T26	37	M	3	9	NV	NA	NS	M(4)
T27	35	M	13	9	V	NA	NS	NM
T28	29	F	10	8	NV	NA	NS	NM
T29	39	M	10	8	NV	NA	NS	NM
T30	49	M	5	6	NV	NA	NS	NM
T31	51	M	32	8	V	NA	NS	M(2)
T32	38	M	5	8	NV	A	NS	NM
T33	37	F	14	4	V	NA	NS	M(4)
T34	25	F	5	7	NV	NA	NS	M(9)

T35	35	F	10	2	NV	NA	NS	NM
T36	49	M	21	8	V	NA	NS	NM
T37	29	F	12	8	NV	NA	NS	NM
T38	36	M	4	4	V	NA	NS	M(1)
T39	37	F	6	5	NV	NA	NS	NM
T40	29	M	3	8	V	NA	S	NM
T41	44	M	11	2	NV	NA	S	NM
T42	33	F	7	7	NV	NA	NS	NM
T43	28	M	2	9	NV	NA	NS	NM
T44	42	M	13	5	V	A	NS	M(3)
T45	52	M	26	3	NV	NA	NS	M(2)
T46	31	F	4	4	V	NA	NS	NM
T47	37	M	17	6	NV	NA	NS	NM
T48	29	F	7	3	V	NA	NS	NM
T49	36	M	14	6	V	NA	S	M(3)
T50	37	F	9	4	V	NA	NS	M(5)

Note: V-vegetarian; NV- non-vegetarian; A-alcoholic; NA- non alcoholic; S- smoker; NS- non smoker; M- micronucleus present (number of micronuclei); NM- micronucleus not present.

Table 4: Descriptive chart for control individuals.

CODE	AGE (years)	SEX	EXPERIENCE (years)	Hours of exposure (h)	Diet V/NV	Alcohol consumption A/NA	Smoking habit S/NS	Micronuclei M/NM
H1	23	M	8	8	NV	NA	NS	M (4)
H2	30	M	7	15	V	NA	NS	M (2)
H3	27	M	2	2	NV	A	NS	M (4)
H4	32	M	19	8	NV	NA	NS	NM
H5	44	F	15	12	V	NA	NS	M (2)
H6	24	M	3	6	V	NA	NS	NM
H7	24	M	8	6	NV	NA	NS	NM
H8	30	F	7	8	NV	NA	NS	NM
H9	26	M	5	8	V	NA	NS	M (1)
H10	25	M	2	8	NV	NA	NS	M (1)
H11	49	M	20	8	NV	NA	NS	NM
H12	32	M	10	9	NV	NA	NS	NM
H13	31	F	8	8	NV	NA	NS	NM
H14	21	F	3	7	NV	NA	NS	NM
H15	24	F	4	8	NV	NA	NS	NM
H16	25	F	11	8	V	NA	NS	NM
H17	21	F	9	6	V	NA	NS	NM
H18	21	F	2	1	V	NA	NS	NM
H19	25	F	4	9	NV	NA	NS	NM
H20	27	F	2	9	V	NA	NS	NM
H21	34	M	7	7	NV	NA	NS	NM
H22	27	F	2	5	NV	NA	NS	NM
H23	31	M	5	8	V	NA	NS	NM

H24	26	F	2	7	NV	NA	NS	NM
H25	25	F	3	8	NV	NA	NS	NM
H26	25	M	6	8	NV	NA	NS	NM
H27	40	F	10	8	V	NA	NS	NM
H28	49	M	20	8	NV	NA	NS	M(2)
H29	20	F	2	8	V	NA	NS	NM
H30	29	F	10	8	NV	NA	NS	NM
H31	32	F	4	8	NV	NA	NS	NM
H32	39	M	6	8	NV	NA	NS	NM
H33	45	M	4	6	NV	A	NS	NM
H34	29	F	3	8	V	NA	NS	NM
H35	29	M	6	10	V	NA	NS	NM
H36	45	F	3	8	NV	NA	NS	NM
H37	47	F	4	8	NV	NA	NS	NM
H38	34	M	6	8	NV	A	NS	NM
H39	39	F	6	8	NV	NA	NS	NM
H40	23	M	2	8	NV	A	NS	NM
H41	36	M	10	9	V	NA	NS	M (1)
H42	38	M	18	9	NV	NA	NS	M (1)
H43	35	M	12	6	NV	A	NS	NM
H44	22	M	2	5	V	NA	NS	NM
H45	34	M	5	9	NV	A	S	M (1)
H46	23	F	2	8	NV	NA	NS	NM
H47	36	F	15	5	NV	NA	NS	NM
H48	52	F	30	7	V	NA	NS	M (1)
H49	30	F	5	8	NV	NA	NS	NM
H50	32	F	8	8	NV	NA	NS	NM

Note: V-vegetarian; NV- non-vegetarian; A-alcoholic; NA- non alcoholic; S- smoker; NS- non smoker; M- micronucleus present (number of micronuclei); NM- micronucleus not present.

Numbers of MN was found to be considerably high in the subject group i.e. chemical laboratory workers as compared to the normal population not exposed to occupational chemicals. The average number of MN observed in the subject group was 1.32 and in control 0.40. In our study the MN in smokers is lesser than in non smokers; and MN number in alcoholics is lesser than in the non alcoholics. The data obtained follows normal distribution for both control and subject groups with statistically significant value ($p<0.05$). There is a significant difference in the means of MN found in control and subject groups. The number of MN in alcoholics, smokers and non-vegetarians are higher in subject individuals than the control. Similarly the number of MN observed in non-smokers, non-alcoholic and vegetarians in higher in subject individuals than the control. Thus this provides evidence that the chemically exposed population bare a risk of genetic damage.

The study indicates that there was a significant increase in the frequency of MN in all subject groups than in the control group: The number of MN observed in subjects was 66 (n=50) and 21 MN in control (n=50) (Fig 2). There was a considerable increase of 9 MN observed in alcoholic subjects (n=5) as against 5 MN seen in alcoholic control. Non-alcoholic subject population exhibited an increase of 37 MN over non-alcoholic control (Fig 4). When the smoking habits of the subjects were taken in consideration the MN frequency was higher in subject than control. The obtained number of MN in non smokers was 57 (n=50) which are higher than the MN observed in the control 19 (n=50) (Fig 5). We have also carried out a study on the dietary habit of subjects and control individuals. The MN frequency in vegetarian subjects is more when compared to control. Similarly for non-vegetarian the MN number is greater in non-vegetarian population of subjects than control (Fig 6).

There is no significant relation found between the experience in the occupation and the time of exposure to laboratory chemicals for the formation of MN.

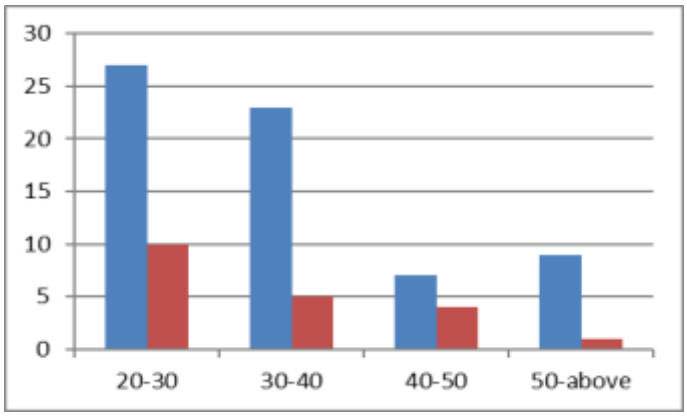

Fig 3: A comparison of the number of MN per 1000 buccal cells in different age groups of control (red) and subject (blue) individuals.

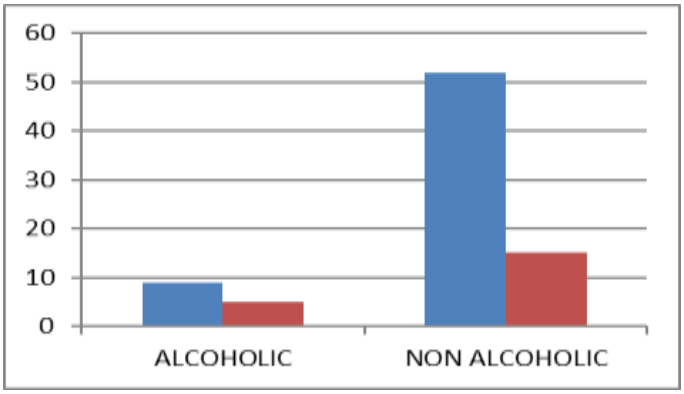

Fig 4: A comparison of the number of MN per 1000 buccal cells in control (red) and subject (blue) individuals differing in alcohol consumption.

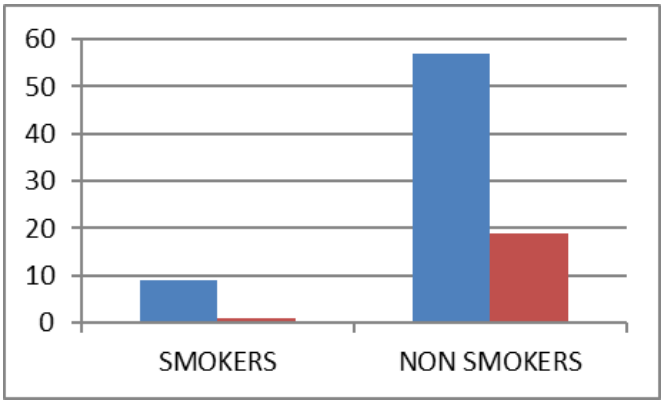

Fig 5: A comparison of the number of MN per 1000 buccal cells in control (red) and subject (blue) individuals differing in smoking habits.

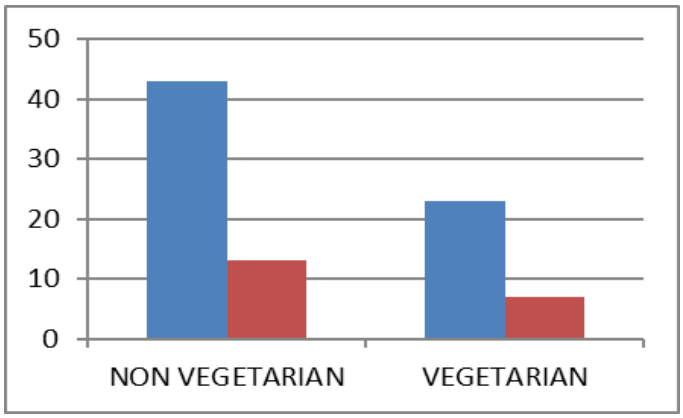

Fig 6: A comparison of the number of MN per 1000 buccal cells in control (red) and subject (blue) individuals differing in dietary habits.

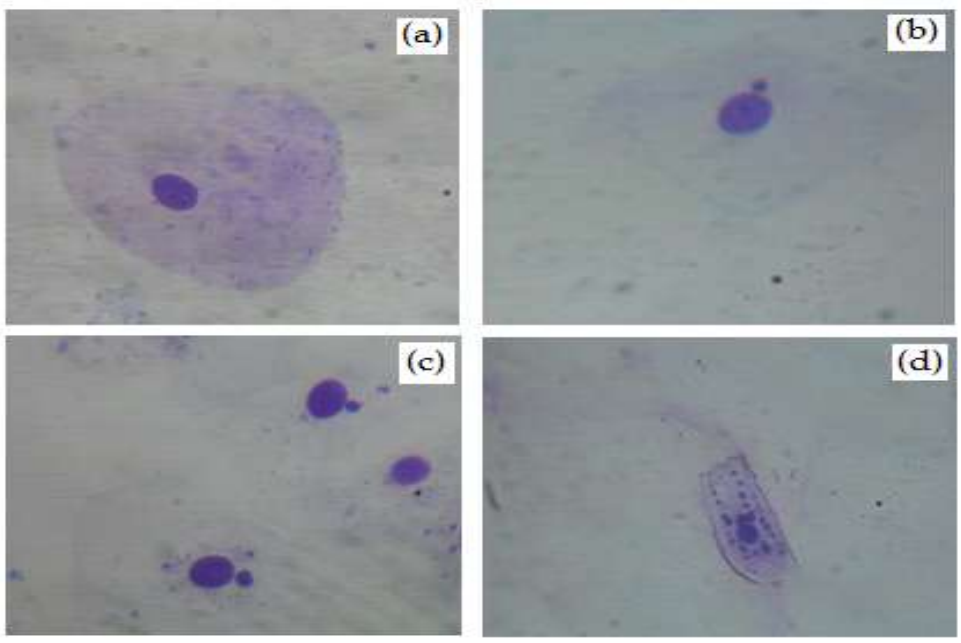

Fig 7: Normal cell without MN (a); single cell with MN (b), (c), (d).

We have observed that most of the chemistry laboratory workers do not take protective measures while handling chemicals, are not well trained and lack basic knowledge about the toxic chemicals present in their laboratory. Although the genotoxic potential of general laboratory chemicals are believed to be comparatively low, the genotoxic monitoring in such population would provide a tool to estimate the risk for long term health effects such as cancer and serious reproductive health outcomes. The genotoxic potential of laboratory chemicals in estimating genetic risk is inclusive of different factors such as age, alcohol consumption and smoking habit other than just the time and duration of exposure. Various literature have reported that all the above factors are significant in occupationally exposed individuals (Tolbert *et al*, 1992).

The MN assay of epithelial cells has emerged as an innovative genotoxicity technique method that correlates well with studies on epithelial carcinoma incidence (Tolbert *et al*, 1992). Previous studies have described the MN assay in buccal cells as a revolutionary technique for monitoring genetic damage in human population (Foiles *et at.*, 1989*;* Sarto *et al.*, 1990*;* Kayal *et al.*, 1993*).* The application of MN test for human biological monitoring can be carried on many cells such as cells of oral mucosa, nasal cavities, oesophagous, urinary tract, cervix etc. for organ specific exposure; and analysis of peripheral blood lymphocytes for total body exposure (Tomanin *et al.,* 1991). According to Heddle (1973), MN assay in exfoliated cells is a simple and convenient test system in which cells can be easily obtained with no requirement of cultivation, comparatively less time consuming, cost effective and can be done with basic laboratory equipments. These micro-nucleated cells are found in the interphase of cell cycle unlike sister chromatid and other chromosomal aberrations.

Cytogenetics is gaining more importance in the coming future due its ability to detect early mutagens/carcinogens, its capability to detect the inheritable changes and its cancer predictability (Battershill, J *et al.*, 2008). Shuhas *et al.*, (2004) observed that the number of MN were higher in beedi smokers than non-smokers, drawing a direct relationship with the development of oral cancer due to the induction of cytogenetic damage in oral epithelial cells. Thus, proving that MN assay can be used as an efficient test in predicting chances of oral carcinoma.

Chemicals are extensively used all over the world and exposure to chemicals remains a major cause of health problems. In India, chemical laboratory worker who are occupationally

exposed to chemicals are generally unaware of the fact that the chemicals which they are getting exposed to directly or indirectly have some toxic effects. The extent of exposure of chemicals is believed to be negligible but accumulation of these chemicals may cause future health problems.

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