In vitro Toxicological Screening of Genetic Damages in Humans by Micronucleus Test

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Abstract: ***A "micronucleus" is a small nucleus. The nucleus is the organelle in the cell that contains the genetic material (DNA) that directs normal cellular function and cellular reproduction. In cells of eukaryotic organisms, the nucleus contains DNA packaged into chromosomes. In vivo mutagencity and carcinogenicity studies are posing a high demand for test related resources. The in vitro micronucleus assay is a mutagenic test system for the detection of chemicals which induce the formation of small membrane bound DNA fragments i.e. micronuclei in the cytoplasm of interphase cells. These micronuclei may originate from acentric fragments (chromosome fragments lacking a centromere) or whole chromosomes which are unable to migrate with the rest of the chromosomes during the anaphase of cell division. The micronucleus assay is an important component of genetic toxicology screening programs. There is substantial evidence that chromosome mutations and related events in oncogenes and tumor suppressor genes of somatic cells are involved in induction and/or progression of some cancers in humans and experimental animals. The study of DNA damage at the chromosome level is an essential part of genetic toxicology because chromosomal mutation is an important event in carcinogenesis. The micronucleus assays have emerged as one of the preferred methods for assessing chromosome damage because they enable both chromosome loss and chromosome breakage to be measured reliably.***

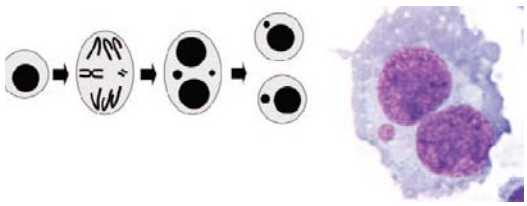
Keywords: Micronucleus, Toxicology, DNA, Screening and Mutagenicity

Introduction

The micronucleus test is an in vivo and *in vitro* short-time screening method is widely used to detect Genotoxic effects. It is one of the simplest, reliable, least expensive and rapid screening system for both clastogenic (chromosome breakage and formation of acentric fragments) and aneugenic (chromosome lagging and effects on spindle) effects. In Anaphase any of the chromosome fragments or whole chromosome which lacks a centromere may not be integrated in the nucleus, because of lack of indispensable elements for orientation in the spindle apparatus. After telophase the fragments or whole chromosome give rise to one or several secondary nuclei which are smaller than the main daughter nucleus and therefore called micro nuclei.[3],[4],[5].

The purpose of the micro nucleus test (MN) to identify substance that cause cytotoxic effect which results in the formation of micronucleus containing lagging chromosome fragments or whole chromosome. Micronucleus are cytoplasmic chromatin containing bodies formed when acentric chromosome fragments or chromosome lag during Anaphase, because enumeration of Micronucleus is much faster and less technically demanding than in scoring of chromosomal aberrations. An increase in the frequency of micronucleated poly chromatic erythrocytes in Animals and indication of induced chromosomal damage, results of Genetic damage that results in chromosomal breaks, structurally abnormal chromosome or spindle abnormalities leads to micro nucleus formation. The current study is aimed to analyze the micronucleus formation in the different student groups, cancer patients and individuals who exposed to alcohols and tobacco smokes.[5],[8],[11].The distinction between these phenomena is important, since the exposure studied often induces only one type of MN. This particularly concerns the use of MN as a biomarker of Genotoxic exposure and effects, where differences in MN frequencies between exposed subjects and referents are expected to be small. A specific analysis of the induced type of MN may considerably improve the sensitivity of detecting the exposure effect.

MN harboring chromosomes can be distinguished from those harboring acentric fragments by the presence of a centromere. [2], [14], [19], [22].The proportion of centromere-positive MN in human lymphocytes increases with age, which primarily reflects an age dependent micro nucleation of the X and Y chromosomes. The X chromosome especially tends to lag behind, in female lymphocyte anaphase, being micronucleated more efficiently than autosomes. There is some evidence for an enhanced prevalence of fragments from chromosome 9 in spontaneous human lymphocyte MN and from chromosomes 1, 9 or 16 in MN induced *in vitro* by some clastogens; the breakage appears to occur in the heterochromatic block of these chromosomes.[17],[18]. Although there are indications that centromere identification can improve the detection of clastogenic effects in human's *in vivo*, smokers have not shown an increase in centromere-negative MN in their cultured lymphocytes, although smoking is known to produce chromosomal aberrations. This may suggest that fragment-containing MN and chromosomal aberrations cover partly different phenomena. Understanding the mechanistic origin and contents of MN is essential for the proper use of this cytogenetic end-point in biomarker studies, genotoxicity testing and risk assessment.



Schematic diagram showing the origin of MN from either a lagging chromosome fragment or a whole chromosome

Materials and Methods

2.1 Blood Sampling

The present study different human populations were selected for sampling. The study groups consist of students like Primary school, high school, pre-university, degree students and cancer patients. Demographic data such as age, sex, profession, locality and other information was recorded. Influence of age, Blood groups and sex in the micronucleus formation is also evaluated. The formation of micronucleus in the different study group was compared and effect of exposure to alcoholics and smokes also evaluated.

2.2 *In Vitro* Micronucleus Test (MNT)

The Capillary blood sample was taken from finger tip of the individuals by pricking with sterilized needle by aseptic precaution and a drop of the blood is placed on the clean slide and thin smear of blood is prepared. Smear is allowed to dry in the air. The smear of blood samples were flooded with Leischmann stain to stain the micronucleus in the cells. Excess of the stain is removed by washing with water. Finally stained smear were observed under the microscope. For the long preservation of the slides are treated with methanol for 20 minutes.

Results and Discussion

The *in vitro* micronucleus assay was performed to analysis of cytotoxicity caused by physiological factors, cancer and alcohol and tobacco smokers. Micronucleus formation is one of the biomarker for genetic damages caused by various factor. After the Leischmann staining with cells micronucleus under microscope is appears as small, pink colored and circular or oval shaped body. In the present finding shows interesting results in micronucleus formation among student populations and more interestingly the alcohol users and smokers showed increased micronucleus formation. In this study high school students observed it was that the micronucleus count is normal except few students (Table 1) by this count it is predicted that in high school students may be less genetic damage when compared to others. Mean micronucleus count was 1.65. Further, there is slightly increase in the micronucleus counts observed in the Pre-university college students (Table 2), the mean micronucleus count is 3.4 and in graduate students still higher count (Table 3) was recorded (Mean micronucleus count is 3.5). This result shows that age is one of the important factors to determine micronucleus count and current findings the results shows gradual increase in each age group is observed. However, results show there is no much variation in micronucleus formation among the sex. The individual who have habits of consuming the alcohols and smoking were found to have increased in micronucleus count, mean micronucleus count is 5.25. It suggests that adverse effect of alcohol and tobacco product on the human cells. It may be due the damage caused by these products on the genetic constituents of the cells. More interestingly cytotoxicity well evidence in the cancer patients where they have high level micronucleus count (Figure 1). The mean micronucleus count in cancer patient is 20.66. The cancer patients showed significantly high number of micronucleus count among the study group, it may be owing to the higher level cytotoxicity or genetic damage usually occurs due to chemotherapeutic or radiotherpeutic effect or immunological responses in these patients.

3.1. **Table 1:** Micronucleus count in the blood smear of the high school students

S. No	*Name*	*Age*	*Sex*	*No. of Micronucleus*
1	HS1	15	Male	4
2	HS2	15	Male	0
3	HS3	15	Male	0
4	HS4	15	Male	0
5	HS5	15	Male	1
6	HS6	15	Male	2
7	HS7	15	Male	1
8	HS8	15	Male	1
9	HS9	15	Male	2
10	HS10	15	Male	3
11	HS11	14	Female	2
12	HS12	14	Female	2
13	HS13	14	Female	2
14	HS14	14	Female	0
15	HS15	14	Female	1
16	HS16	14	Female	3
17	HS17	14	Female	1
18	HS18	14	Female	2
19	HS19	14	Female	3
20	HS20	14	Female	3

3.2 **Table 2.** Micronucleus count in the blood smear of the Pre-university students

S. No	*Name*	*Age*	*Sex*	*No. of Micronucleus*
1	PU1	18	Male	4
2	PU2	18	Male	0
3	PU3	18	Male	5
4	PU4	18	Male	4
5	PU5	18	Male	6
6	PU6	18	Male	3
7	PU7	18	Male	4
8	PU8	18	Male	4
9	PU9	18	Male	3
10	PU10	18	Male	0
11	PU11	17	Female	4
12	PU12	17	Female	6
13	PU13	17	Female	2
14	PU14	17	Female	3
15	PU15	17	Female	4
16	PU16	17	Female	3
17	PU17	17	Female	0
18	PU18	17	Female	3
19	PU19	17	Female	3
20	PU20	17	Female	7

3.3 Table 3: Micronucleus count in the blood smear of the graduate students

S. No.	*Name*	*Age*	*Sex*	*No. of Micronucleus*
1	DS1	20	Male	4
2	DS2	20	Male	4
3	DS3	20	Male	5
4	DS4	20	Male	3
5	DS5	20	Male	4
6	DS6	20	Male	5
7	DS7	20	Male	3
8	DS8	20	Male	3
9	DS9	20	Male	3
10	DS10	20	Male	4
11	DS1	19	Female	3
12	DS12	19	Female	3
13	DS13	19	Female	2
14	DS14	19	Female	3
15	DS15	19	Female	4
16	DS16	19	Female	3
17	DS17	19	Female	4
18	DS18	19	Female	3
19	DS19	19	Female	4
20	DS20	19	Female	3

3.4 Table 4. Micronucleus count in the blood smear of the alcohol drinker and tobacco smoker

S. No	*Name*	*Age*	*Sex*	*No. of Micronucleus*
1	SD1	19	Male	7
2	SD2	22	Male	9
3	SD3	26	Male	5
4	SD4	24	Male	6
5	SD5	31	Male	4
6	SD6	35	Male	2
7	SD7	36	Male	9
8	SD8	32	Male	8
9	SD9	31	Male	4
10	SD10	30	Male	5
11	SD1	28	Male	6
12	SD12	39	Male	2
13	SD13	40	Male	3
14	SD14	26	Male	1
15	SD15	29	Male	6
16	SD16	29	Male	4
17	SD17	30	Male	9
18	SD18	32	Male	7
19	SD19	32	Male	3
20	SD20	36	Male	5

3.5. Table 5. Micronucleus count in the blood smear of the cancer patients

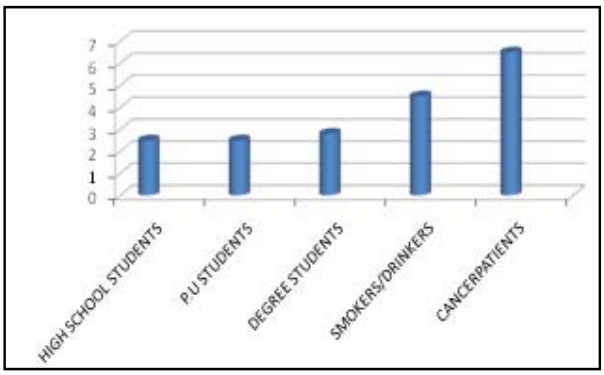
S. No	*Name*	*Age*	*Sex*	*No. of Micronucleus*
1	Patient1	38	Male	18
2	Patient2	40	Male	19
3	Patient3	41	Male	20
4	Patient4	42	Male	23
5	Patient5	44	Male	28
6	Patient6	48	Male	25
7	Patient7	49	Male	26
8	Patient8	50	Male	24
9	Patient9	52	Male	16
10	Patient10	54	Male	18
11	Patient11	56	Male	28
12	Patient12	56	Male	15
13	Patient13	58	Male	17
14	Patient14	58	Male	19
15	Patient15	59	Male	14

3.6 A Detail Study Of Micronucleus Count In Under Graduate Students With Reference To Blood Group:

S No.	*Student Name*	*Age*	*Sex*	*Blood Group*	*Micronucleus Count*
1	S1	18	Male	A+	2
2	S2	18	Male	A+	2
3	S3	19	Male	A+	3
4	S4	18	Male	A+	2
5	S5	17	Male	A+	0
6	S6	19	Male	A-	2
7	S7	18	Male	A-	0
8	S8	18	Male	A-	2
9	S9	21	Male	A-	4
10	S10	18	Male	A-	2
11	S11	20	Male	B+	1
12	S12	20	Male	B+	2
13	S13	20	Male	B+	1
14	S14	20	Male	B+	2
15	S15	20	Male	B+	3
16	S16	20	Male	B-	2
17	S17	20	Male	B-	4
18	S18	21	Male	B-	2
19	S19	21	Male	B-	2
20	S20	20	Male	B-	2
21	S21	21	Male	O+	2
22	S22	19	Male	O+	1
23	S23	19	Male	O+	2
24	S24	20	Male	O+	3
25	S25	20	Male	O+	0
26	S26	19	Male	O+	0
27	S27	18	Male	O+	1
28	S28	20	Male	O+	1
29	S29	18	Male	O+	2
30	S30	20	Male	O+	1
31	S31	20	Male	O+	2
32	S32	20	Male	O+	3
33	S33	20	Male	O+	3
34	S34	20	Male	O+	2
35	S35	20	Male	O+	2
36	S36	20	Male	O+	2
37	S37	20	Male	O+	2
38	S38	20	Male	O+	1
39	S39	20	Male	O+	3
40	S40	20	Male	O+	4
41	S41	19	Male	O-	2
42	S42	19	Male	O-	1
43	S43	18	Male	O-	1
44	S44	17	Male	O-	1
45	S45	17	Male	O-	3
46	S46	18	Male	O-	2
47	S47	19	Male	O-	2
48	S48	19	Male	O-	2
49	S49	18	Male	O-	1
50	S50	19	Male	O-	3
51	S51	18	Female	A+	4
52	S52	18	Female	A+	2
53	S53	18	Female	A+	2
54	S54	18	Female	A+	3
55	S55	19	Female	A+	3
56	S56	19	Female	A+	4
57	S57	19	Female	A+	1
58	S58	18	Female	A+	1
59	S59	17	Female	A+	1
60	S60	18	Female	A+	1
61	S61	18	Female	A-	0
62	S62	18	Female	A-	2
63	S63	18	Female	A-	2

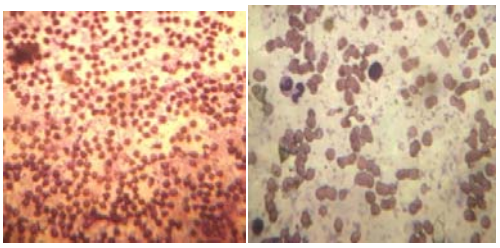
64	S64	18	Female	A-	0
65	S65	18	Female	A-	0
66	S66	18	Female	B+	0
67	S67	20	Female	B+	0
68	S68	20	Female	B+	0
69	S69	20	Female	B+	2
70	S70	1	Female	B+	1
71	S71	20	Female	B-	2
72	S72	20	Female	B-	1
73	S73	20	Female	B-	1
74	S74	19	Female	B-	2
75	S75	18	Female	B-	1
76	S76	20	Female	O+	1
77	S77	19	Female	O+	1
78	S78	19	Female	O+	2
79	S79	20	Female	O+	1
80	S80	19	Female	O+	2
81	S81	19	Female	O+	1
82	S82	19	Female	O+	3
83	S83	19	Female	O+	0
84	S84	18	Female	O+	0
85	S85	20	Female	O+	2
86	S86	19	Female	O+	2
87	S87	19	Female	O+	1
88	S88	19	Female	O+	3
89	S89	20	Female	O+	4
90	S90	19	Female	O+	1
91	S91	21	Female	AB+	2
92	S92	21	Female	AB+	2
93	S93	19	Female	AB+	2
94	S94	18	Female	AB+	2
95	S95	18	Female	AB+	3
96	S96	19	Female	O-	1
97	S97	20	Female	O-	1
98	S98	19	Female	O-	1
99	S99	19	Female	O-	2
100	S100	18	Female	O-	1

3.7 Figure 1: Comparison of Micronucleus count among Study Group



By the above graph we conclude that in Cancer patients maximum number of micronucleus count is noticed than compare to the High school students, Degree students and in Smokers.

Photographs



Micronucleus in Blood Smear

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