World Journal of
Experimental Biosciences

Volume 4, Number 1: 40-48.
ISSN: 2313-3937
2016

Research article

Frequency of micronuclei in peripheral blood lymphocytes of healthy individuals: A study from Baghdad, Iraq

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ABSTRACT

Monitoring cytogenetic damage is frequently used to asses population exposure to environmental mutagens. The cytokinesis block micronucleus assay is one of the most widely used methods employed in these studies because it offers simultaneous information on DNA damage and cytotoxic/cytostatic effects caused by possible genotoxic agents. The present study was designed to assess the genotoxic and cytotoxic damage in peripheral blood lymphocyte of individuals residing in two different districts in Baghdad assuming that both of them are exposed to high level of pollutant this is done by detecting micronuclei in binucleated lymphocyte and nuclear division index. The study population consisted of 163 apparently healthy . One hundred –three individuals, including (41 males and 62 females) from district 1 (Jeser Diyala) and 60 individuals from district 2 (Alhurriya) including 35 males and 25 females. The age of study participant ranged from 15 to 46 years at the time of inclusion. The mean count of binucleated lymphocytes with micronuclei (BNMN) in the total sample was 24.43±19.66. The mean count of BNMN in district 1 was 31.63±20.76 and in district 2 was 12.32±8.91. The difference between two districts was significant (P< 0.0001). The mean value of the nuclear division index (NDI) in total samples was 1.11±0.12. In district 1 was 1.14±0.14, which was significantly higher than NDI in district 2 individuals (1.07±0.06), (P< 0.001). The present study concluded that Individuals participating in the present study have been exposed to an action of one or more micronuclei inducing agents that elicit high BNMN mean count especially in district 1.While the reduction of nuclear division index in the studied sample particularly in district 2 could be due to exposed to an agent that have cytotoxic properties affecting the cell proliferation kinetics and considered as an early marker of cytotoxicity and genotoxicity. This work reveals many background data that may be of value in future genotoxicological monitoring studies in Iraq.

Keywords: BNMN, NDI, Lymphocytes, Iraq.

Citation: **Shafi FAA, AL-Ansari N, Abdul Majeed BA.** (2016) Frequency of Micronuclei in Peripheral Blood Lymphocytes of Healthy Individuals: A study from Baghdad Iraq. *World J Exp Biosci* **4**: 40-48.

Received January 3, 2016; Accepted February 2, 2016; Published February 10, 2016.

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INTRODUCTION

Humans are constantly exposed to variety of natural and synthetic genotoxic substance, by reaction with cellular biomolcules (DNA, RNA and protein), genetic alterations such as chromosomal aberration and formation of micronuclei in cells, are the early biological effects which may lead to an increased cancer risk or aging [1,2] many studies reveled that individuals difference in response to mutagenic challenge and the actual values genetic damage can partly be influenced by endogenous factors including those of biological origins such as gender, age and white blood cell count. A variety of external factors such as chemicals, physical agents, viruses, life-style (smoking and drinking habits, nutrition), residential and/or working areas [3,4,5].

In view of these findings with advances in molecular biology, many studies have been established for monitoring population exposed to known or suspected mutagen by using cytogenetic biomarkers as biomarkers of effects and exposure with combination with susceptibility biomarkers to identify a high-risk individuals and prevent adverse health effects [6,7]. Micronuclei assay may contribute to a better understanding of these mechanisms since this is a surrogate biomarker of chromosome breakage/or loss, genetic instability and/or exposure to environmental mutagens or carcinogens. Occurrence of MN in peripheral blood lymphocytes (PBLs) has been associated with cancer risk in human [5, 8-11]. Micronuclei (MN) are chromatin-staining structures in the cytoplasm surrounded by a membrane without any detectable link to the cell nucleus visualized using different staining techniques (Giemsa or fluorescent) and their frequency is quantified microscopically [12]. The formation of (MN) in dividing cells is the result of chromosome breakage due to unrepaired or mis-repaired DNA lesions, or chromosome malsegregation due to mitotic malfunction. These events may be induced by oxidative stress, exposure to clastogens or aneugens, genetic defects in cell cycle checkpoint and/or DNA repair genes, as well as deficiencies in nutrients required as cofactors in DNA metabolism and chromosome segregation machinery [13]. The present study was designed to assess the genotoxic and cytotoxic damage in peripheral blood lymphocyte of individuals residing in two different districts in Baghdad assuming that both of them are exposed to high level of pollutant. This is done by detecting MN in binucleated lymphocyte and nuclear division index (NDI) to investigate if there is a relation with external factor such as life style habits and various occupational and environmental exposures and internal factors (age and gender).

MATERIALS AND METHODS

Study population

The samples collection and practical work of the present study extended through the period from October 2009 to June 2010.

The study population consisted of 163 apparently healthy individuals from two districts in Baghdad. One hundred–three individuals including (41 males and 62 females) from district 1 (Jeser Diyala) and 60 individuals from district 2 (Alhurriya) including 35 males and 25 females. The age of volunteers ranged from 15 to 46 years at the time of study. Age, marital status, smoking habit, alcohol consumption, diagnostic X rays, chemical exposure during occupation, family history of cancer, medical and residential history were collected by questionnaire. All analyses were carried out on anonymous, coded samples.

Cytokinesis blocked micronucleus (CBMN) assay

heparinized blood samples collected by venipuncture were obtained from each individuals and used to culture peripheral blood lymphocytes. Cytokinesis blocked micronucleus (CBMN) assay was performed as described by Fenech and Morely [14]; berifely, lymphocyte culture was set up by adding 0.5 ml of whole blood to 4.5 ml of RPMI 1640 (Sigma) medium supplemented with 20% heat inactivated fetal calf serum (Gibco). Lymphocytes were stimulated with (0.1 ml) PHA (Sigma) and incubated at 37°C. Two cultures preparation were made for each individual.

Cyto-B(Sigma) was added to the cultures (44 h old) in a final dilution, 6 µl/ml to arrest cytokinesis. After 72 h of incubation, the cultured cells were harvested by centrifugation at 1000 rpm for 5 min. The supernatant was discarded and cell pellets were treated with a hypotonic solution (0.075 M KCL) for 5 min. After centrifugation at 1000 rpm for 5 min, the cells were fixed in fresh fixative (methanol/glacial acetic acid, 3:1). The fixative was then removed by centrifugation this fixation step was repeated twice, and the resulting cells were resuspended in a small volume of fixative. Staining of slide done by dropping cell suspension using a Pasteur pipette, on to cleaned, grease-free, labeled microscope slides that had been pre-dipped in cold distilled water. Drops were air-dried, stained with Giemsa stain (BDH) (25%) for 2 min and examined with a light microscope using oil immersion magnification. Scoring Procedure was done by following the criteria proposed by Fenech [15]. A total of 1000 binucleated cells with well preserved cytoplasm were examined per 10 high power fields for the presence of micronuclei. The mean counts was taken for each individuals as number of binucleated lymphocytes with micronuclei/1000 binucleated lymphocytes. The mean counts were categorized into the following score groups according to Fenech [16] (negative, no micronuclei; low score, 1-6 BNMN/ 1000; intermediate score, 7-30 BNMN/1000; high score, 31-80 BNMN/1000).

Nuclear division index (NDI) was calculated according to Surralles et al [17]. NDI can be calculated by the following equation,(1N+2×2N+3×3N+4×4N)/500 cells (1N, number of cells with one nucleus; 2N, number of

cells with two nuclei; 3N, number of cells with three nuclei; 4N, number of cells with four nuclei.

Statistical analysis

All statistical analysis were conducted using an SPSS (version 11). A statistical test included, Person χ^2 test was used to test the difference between two populations in term of sex (between females and males) and between occupations, family history of cancer smoking habits, abortion. t test was used to analyze the difference in mean age also to detect difference in the mean of BNMN and NDI between studied population. A Person's correlation coefficient analysis was calculated to establish possible relationship between BNMN and NDI. Person chi square test was used to detect difference in BNMN score. The effect of age and occupation on BNMN and NDI were evaluated by Kreskuall Wallas test. The Mann-Whitny U-test was used to test the difference in mean of BNMN and NDI between male and female, smoker and nonsmoker. P value regarded significant at P < 0.05 for each test.

RESULTS

Demographic characteristics of the studied population

The demographic characteristics of the studied population are summarized in **table 1**. Individuals in both districts had the same mean age (24.80 ± 7.44, 24.23±6.5). The difference between two districts regarding the mean age was statistically not significant (P>0.05). Females in district 1 (Jeser Diala) constituted 60.2% (62).Those in district 2 (AL-Hurriyah) constituted 41.7% (25). The percentages of males were 39.8% and 58.3 % for district 1 and district 2, respectively. The difference between individuals in two districts regarding percentage of female and male was statistically significant (P< 0.05). Fifty eight of female in the study group were housewives. Occupations of all study group are listed in **table 1**.

Individuals in district 1 significantly (P< 0.05) differed from individuals in district 2 with respect to the occupation and marital status. Married individuals in district 1 had higher proportion of abortion 26% (18) compared to the those in district 2, 7% (1). Smokers comprised 12.6% (13) of individuals in district 1 and 8.3% (5)of individuals in district 2. All study participant had no history of alcohol consumption. Nine individuals (8.7%) of district 1 self-reported a family history of cancer affecting the first degree relatives, compared with 7 individuals (11.7%) of the individuals of district 2. The difference between the study individuals in two districts regarding smoking habits and family history of cancer was statistically not significant (P>0.05).

Table 1. Baseline characteristics of study populations.

	District1 N, 103	District 2 N, 60	Total
Gender	N(%)	N(%)	N(%)
Female	62(60.2)	25(41.7)	87(53.4)
Male	41(39.8)	35(58.3)	76(46.6)
P (Pearson x^2) 0.02			
Age (years) Mean ±SD(range)	24.80±7.44 (15-45)	24.23±6.58 (15-46)	24.59±7.12 (15-46)
P *(t-test) 0.62*			
Housewife	58 (56.3%)	12 (20%)	70
Student	21 (20.38%)	14 (23.3%)	35
Employee	7 (6.79%)	11 (18.3%)	18
Other	17 (16.5%)	23 (38.3%)	40
P(Pearson x^2) 0.000			
Smoker	13(12.6%)	5(8.3%)	18 (11.0%)
Non smoker	90(87.4%)	55(91.7%)	145 (89%)
P (Pearson *x^2*)0.40			
Single	34 (33.0%)	47 (78.3%)	81 (49.7%)
Married	69 (67.0%)	13 (21.7%)	82 (50.3%)
P *(Pearson x^2)*0.000			
Family history of cancer			
yes	9 (8.7%)	7(11.7%)	16 (9.8%)
no	94 (91.3%)	53(88.3%)	147 (90.2%)
P *(Pearson x^2)* 0.5			
Abortion			
Yes	18 (17.5%)	1 (1.7%)	19 (11.7%)
No	51 (82%)	12 (98.3%)	144 (88.3%
P (0.002)			

BNMN) and NDI scoring

Table 2 summarizes mean count of BNMN cells/1000 binucleated lymphocyte (**Fig.1**) and NDI mean value of 101 individuals (60 female,41 male) from district 1 and 60 individuals (25 female,35 male) from district 2. BNMN and NDI were not included for two individuals in district 1 because the blood sample volume was very small). The mean count of BNMN in total sample was 24.43±19.66. The mean count of (BNMN) in district 1 was 31.63±20.76 (ranged between 0 and 80 BNMN/1000 binucleated lymphocyte). In district 2 it ranged between 0 and 40 BNMN/1000 Binucleated lymphocyte with a mean of 12.32±8.91. A significant two-fold increase in genetic damage rate for the individuals of district 1 to the districts 2 individuals was noticed (P <0.0001).

The mean of NDI for lymphocytes in total sample was 1.11±0.12). In districts 2 was 1.07±0.06, which was significantly lower than NDI in districts 1 individuals (1.14±0.14), (P< 0.001). A positive correlation between BNMN and NDI in district 1 (r = 0.22; P, 0.026) was noticed. While, there was negative correlation between BNMN and NDI in district 2 (r, -0.18; P, 0.16).

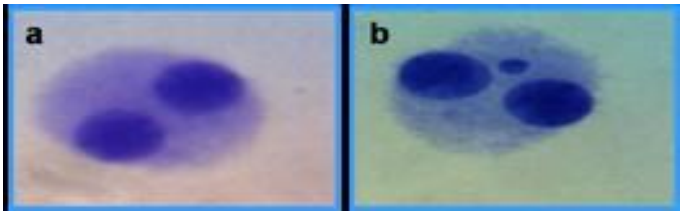

Fig 1. Microscopically 1000X magnitude image of binucleated cell in Giemsa stain A: normal binucleated lymphocytes. B, binucleated cell with micronucleus (MN).

Table 2. Mean count of BNMN/1000 binucleated lymphocytes and NDI in study sample. ***, P< 0.0001; **, P<0.001 (t- test) when compared with district 2; [a] (range). r, - 0.22; P, 0.026 district 1; r, -0.18; P, 0.16 district 2.

Study sample	N	BNMN mean±SD	NDI mean ±SD
Total sample	161	24.43±19.66 (0-80)[a]	1.11±0.12 (1.01-2.00)[a]
District 1	101	31.63± 20.76*** (0-80)	1.14±0.14** (1.01-2.00)
District 2	60	12.32±8.91 (0-40)	1.07± 0.06 (1.01-1.33)

Table 3. Mean count of BNMN /1000 binucleated lymphocytes and NDI by gender. *, P>0.05 female vs male in district 1 (mann-whiteny Utest).

Test type	District 1		District 2	
	Female N, 60	Male N, 41	Female N, z5	Male N, 35
BNMN mean ±SD	32.7± 21.16	29.98±20.30	13.08±9.30	11.77±8.72
NDI mean ±SD	1.17±0.17	1.08±0.07*	1.07±0.06	1.07±0.07

Categories of BNMN score (district 1 and district 2).

Fig 2 depicts score categories of BNMN in studied sample classified into low (1-6), intermediate (7-30) and high score (31-80) according to Fenech [16]. Low score was found in 5 individuals (4.9%) of districts 1 and 20 (33.3%) of districts 2 individuals. Intermediate score was found in 47.5% (48) and 63.3% (38) individuals of district 1 and 2, respectively. There was no significant difference between two districts regarding intermediated score (P>0.05). There was a significant difference between two districts regarding high score (P<0.0001). High score was found in 47.5% (48) and 3.3% (2) individuals of districts 1 and 2, respectively.

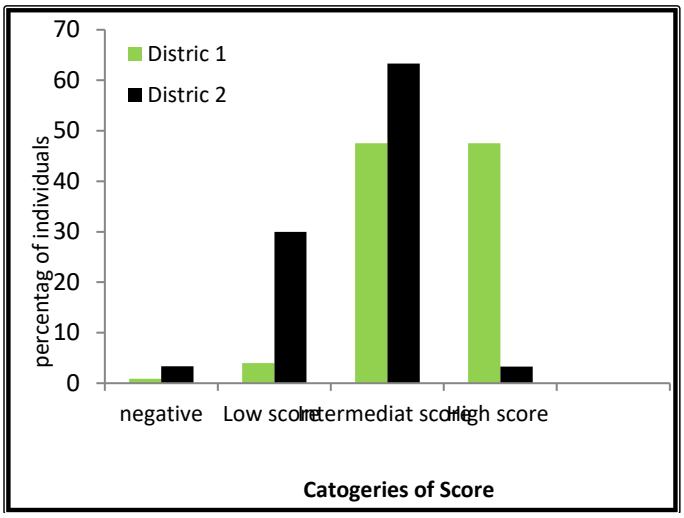

Fig 2. Categories of BNMN score of study sample(district 1 and district 2).

The relation of gender to BNMN mean count and NDI

Females showed a slightly higher BNMN (32.77±21.16; 13.08±9.30 of district 1 and 2, respectively) when compared to males (13.08±9.30; 11.77±8.72 of district 1 and 2, respectively). The difference was statistically not significant (P> 0.05). Males in district 1 had lower NDI (1.08±0.07) compared to females (1.17±0. 17). There was a significant statistical difference between male and female in district 1 regarding NDI (P < 0.05) (**Table 3**).

The relation of age to BNMN mean count and NDI

Fig 3. depicts the relationship between mean count of BNMN and age groups in sample study (districts 1 and districts 2). Within districts 1 higher mean count of BNMN was found among subjects with age group of 26-36 (34.97±19.9) when compared to subjects of age group 15-25 and > 36 (30.02±21.88; 3.00±0.04). However, the difference did not seem to be statistically significant. Whereas in districts 2 subjects the mean count of BNMN decrease in order from the youngest(15-25) to the oldest age group (26-36) (from 12.93±9.93 to 9.33±4.16, respectively). The difference between the groups was statically not significant (**Fig 4**). The mean of NDI in district 2 individuals increased in order from the youngest age group (15-25) to the oldest age group (from1.06±0.05 to 1.11±0.02 respectively). In districts 1 individuals the mean value of NDI in oldest age groupe (≥ 36) was (1.06 ±0.04) it was lower than the mean value in youngest age group (1.14±0.16; 1.15±0.12) for 15-25 and 26-36 age groups, respectively. The difference was statistically not significant

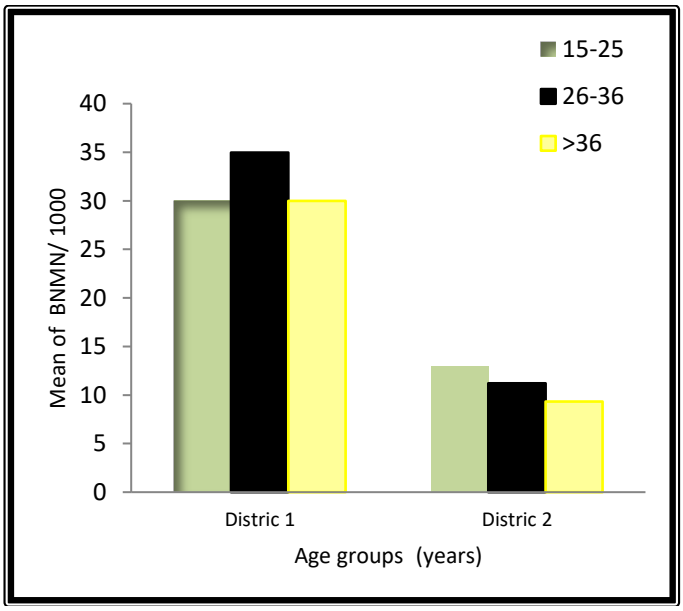

Fig 3. Mean count of binucleated lymphocyte BNMN /1000 in study population according to the age groups.

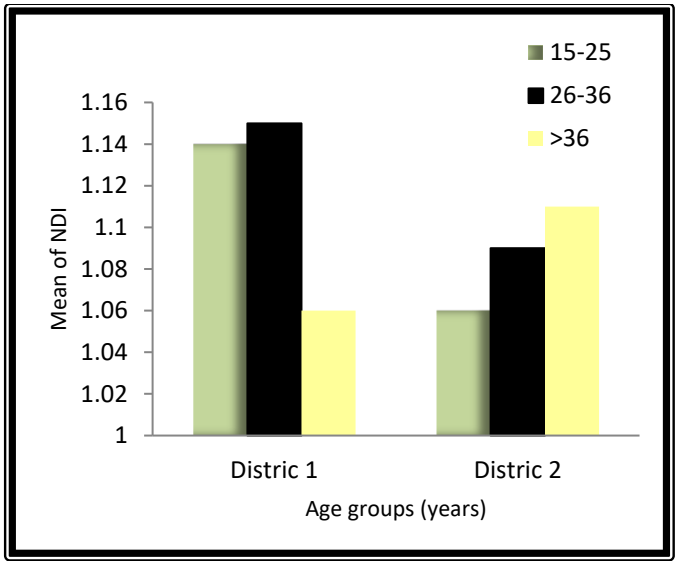

Fig 4. Mean of NDI in study population according to the age groups.

The relation of occupation to BNMN and NDI

As it seen in **table 4** study participant were classified into four groups according to type of occupation (housewives, student, employee and others). In district 1 their mean count of BNMN were 32.46±21.13, 30.95±21.79, 28.14±18.44 and 31.12±20.79, respectively. Their NDI mean value were 1.17±0.17; 1.09±0.07; 1.14±0.14 and 1.07±0.04 . There was a significant difference within groups of district 1 regarding NDI (P< 0.05). The majority of individuals 38% (23) in district 2 were included in group 4 (other) their mean of BNMN was (12.65±9.77), and their NDI mean was (1.06±0.06).There was no significant difference within groups of district 2 regarding BNMN and NDI. (P> 0.05).

Table 4. The mean ± SD of BNMN per 1000 binucleted lymphocyte and NDI in study population distributed according to the occupation. *, P <0.05 (Kruskal wallis test).

	District 1 (N, 101)			District 2 (N, 60)		
	N(%)	BNMN	NDI	N(%)	BNMN	NDI
House wives	57 (56.4%)	32.46±21.13	1.17±0.17	12(20%)	10.92±9.49	1.08±0.07
Student	20 (19.8%)	30.95±21.79	1.09±0.07	14(23.3%)	14.36±9.33	1.05±0.034
employee	7 (6.9%)	28.14±18.44	1.14±0.14	11(18.3%)	10.55±5.87	1.10±0.08
Other	17(16.8%)	31.12±20.79	1.07±0.04*	23(38.3%	12.65±9.77	1.06±0.06

The relation of smoking habits to BNMN mean count and NDI

Most individuals in the two districts were non –smokers. Smokers constituted 12% (13) and 8% (5) of district 1 and district 2 individuals, respectively. Their means ±SD. of BNMN were 32.00±20.58, 10.60±1.67 of district 1 and district 2, respectively. Their means of NDI were 1.11±0.11 and 1.11±0.10 of district 1 and district 2, respectively. The differences in mean count of BNMN and NDI between smokers and non smokers were statistically not significant in both districts (P> 0.05) (**Table 5**).

Table 5. Mean ±SD of BNMN and NDI in study sample according to smoking habits.

	District 1			District 2		
	N (%)	BNMN/1000	NDI	N (%)	BNMN/1000	NDI
Smoker	13 (12.6%)	32.00±20.58	1.11 ± 0.11	5 (8.3%)	10.60±1.67	1.11 ± 0.10
Non smoker	88 (85.4%)	31.58± 20.90	1.14 ± 0.15	55 (91.6%)	12.47±9.29	1.06 ± 0.06

DISCUSSION

This study has revealed that the mean count of BNMN in sample study (24.43± 19.66) is substantially higher than basline value for the general population (6.5 / 1000 BNMN in human standardization study, which is considered basline value for general population) [18] (**Table 2**). It also exceeded above the baseline data that are reported in several countries. The baseline mean count of BNMN per 1000 binucleted cell is 4.74± 0.31 in Croatian [19], 9.16 ±1.0 in Yugoslavian [20], 5 in Japanese [21] and 6.5 in Spain [5]. However, this elevation in the mean count of BNMN observed in this group of local population has not been previously reported. The mean count of BNMN reported in the present study markedly is higher than those reported by other studies on control individuals of Iraqi population. AL-Zayadi [22] and Alwan [23] have reported that BNMN mean count is 6/ 1000 and 5/ 1000 binucleated lymphocyte respectively. Whereas, AL-Ramahi [24] was unable to detect any BNMN in the control group.

This difference may be explained by either different exposure conditions or by demographic factors, individual habits and associated genetic features. Therefore, Anderson [25] suggests that each biomontaring study is unique and in order to estimate the effects, each population should be studied separately and results should not be generalized. The most impressive result of this study is the increased mean count of (BNMN) in peripheral blood lymphocyte of district 1 individuals (mean count of BNMN is 31.63). This increase is statistically significant (P<0.0001) when compared to district 2 individuals (mean count 12.32) (**Table 2**). The present study indicate that individuals of district 1 are exposed to the action of one or more micronuclei inducing agents. Subscribe previous studies In his study Nabyle [26] reported that AL-Twaitha region exposed to low dose of radioactive materials. Afterward AL-Zayadi, [27] reported that the population of AL-

Twaitha region (including district 1 in present study) showed higher frequency of HPRT gene mutation. HPRT gene mutation is considered highly efficient bioindicators to evaluate individuals and group irradiation. Moreover, district 1 is located near the most polluted river (diyala river) [28] which makes human population in this district exposed to potential hazardous substances. Similarly, the mean count of BNMN in this study was comparable to that reported by research on population exposed to diverse genotoxic agents. Other invistegator reported that people living in building constructed with cobalt-60 contaminated steel rods had mean count of 30 BNMN/1000 [29]. The same results were obtained in other studies included children in Belarus from radionuclide contaminated region as result of Chernobyl catastrophe (mean count of 15-45 BNMN/1000) [30]. Individuals who were exposed to occupational or environmental radiation had mean count of 7.95± 5.95 [31]. A Bulagarian study included 103 individuals exposed to lead with mean count of BNMN 42.69 [32]. Stoia et al. [33] demonstrated that the ability of inorganic lead to induce genetic response comparable to that induced by cumulative radiation over an individuals working lifetime. Workers in shoes factory exposed to air contained high concentration of various organic solvent had mean count of 31.13-45 BNMN /1000 Binucleated lymphocytes [34].

In present study, the value of NDI was 1.14±0.14, 1.07±0.06 for districts 1and 2, respectively. NDI value in district 2 was significantly lower than those found in district 1 ($P < 0.001$) (**Table 2**). The value of these indexes in healthy individuals unexposed to toxic substances is very near to 2 or slightly higher. In present study, NDI was 1.14±0.14, 1.07±0.06 for districts 1 and 2, respectively. El-Zein et al [35] and Ezquerro et al. [36] when evaluating genotoxic agents found NDI values for controls higher than those found in this study (2.8± 0.01 and 1.55±0.27, respectively).

Impairment of lymphocyte proliferation measured as NDI reflects inhibiting of the cell cycle progression and/or loss of proliferative capacity and is considered as an early marker of cytotoxicity and genotoxicity and may translate into reduced resistance to disease [37-39].

There are many hypotheses to explain lower NDI in sample study. Firstly, circulating lymphocytes under the toxic effect of mutagenic agents suffer DNA damages and cannot survive the cell cycle. Instead, they enter in a process of apoptosis before the end of the first division or cell select to die before cell culture. Secondly, cell with DNA damage postponement the cell cycle in order to repair the damage and avoid the fixation of mutation during replication, will modify the number of the cells entering mitosis and modify the proportion of mono-/bi-/tri- and tetranucleated cells. Thus, a lower NDI as fewer cells divide. Thirdly, there is the hypothesis of a clastogenic effect of mutagens with an aneugenic action, inducing blockade of the cell cycle. Therefore, more cells will not divide and NDI will again be low [40,41].

The reduction of NDI may support the hypothesis that Individuals in two districts were exposed to high levels of environmental pollution [40]. However, the fact that BNMN mean count was higher in district 1 individuals compared to those in district 2 individuals shows that the repair may not be efficient to correct the mutation caused by exposure. It may likewise indicate that district 1 individuals were exposed to genotoxic agents that caused chromosomal damage without killing the cells, the fact that the surviving cells are perpetually undergoing important mutations demonstrated by the increased score of BNMN. It is important to note that the probability of micronucleted lymphocyte completing division in vitro after PHA stimulation may be much less than that of non- micronucleted lymphocyte. Sablina et al. [42] suggested a p53 –mediated cell cycle checkpoint preventing proliferation of micronucleted cells and that abolition of a p53 function allowed micronucleted cells to complete nuclear division. The number of MN in lymphocyte depends on the proportion of cells that have responded to the mitogen and proportion of the responding cells that have divided more than once. Individuals genotoxic and cytotoxic response led to reduce the dividing rate of lymphocyte and therefore, hinder MN expression and observation this explanation supported by positive correlation coefficient between BNMN and NDI in district 1 (r, 0.22; P, 0.026).

In contrary to district 1, there was negative non significant correlation between BNMN and NDI in district 2 (r, -0.18; P, 0.16). It is important to refer to that since measurement of DNA damage is restricted to MN in binucleated cells. non dividing cells and some of the damage cells ,on the other hand may tend to undergo necrosis or apoptosis instead of dividing. Individuals districts 2 could have been exposed to agents with oxidative properties which may induce stress in cells in contact with them Effects of oxidative stress on cells are include reduction of cellular proliferation , induction of sister chromated exchange (SCE) and chromosomal aberrations [43,44]. Furthermore, oxidative stress causes decrease in the lymphocyte response to mitogenic agents [45]. In **fig 2** regarding to BNMN most individuals in district 1 had intermediate and high scores. In contrary, individuals in district 2 had low and intermediate scores.

In present study, females had slightly higher BNMN mean count than males. However, the difference was not statistically significant (**Table 3**). While no ages related increase in BNMN mean count was detected in study samples (**Fig 3**).This could be due to small number of older individuals since most of the ages were within the 15-25 years resulting in a lower mean age for each district (24 years). The results obtained in this study are in good agreement with results reported by El-Zein et al. [46] and Ezquerro et al. [36]. Neri et al. [47] suggested that gender effect becomes more pronounced with increase age. The large pooled datasets coming from international collaborative studies on biomarkers has allowed evaluation of the role of gender in a more

systematic way. Evaluation of the data showed that BNMN frequency increases monotonically with age in both genders, with the steepest increase after 30 years of age. On average, females were shown to have a 19% higher level of BNMN frequency compared to males. A remarkable increase of BNMN frequency in females starting in the age class 20–29 and increasing progressively [18].

No specific effect of age on proliferative ability of lymphocyte (NDI) was observed in two districts (**Fig 3**). These results are similar to other human studies which reported no variation in lymphocyte proliferative rate with age in controls from the united Kingdom [48]. Similarly sex-related difference in mitogen induced lymphocyte proliferation observed in district 1 were similar to others [49, 19].

To further investigate the possible modulation of genotoxic effects by occupational exposure the individuals in the study were distributed into four groups housewives, students, emplyee and others (**Table 4**). There was a significant difference in mean value of (NDI) within groups in districts 1. No significant difference considering mean count of BNMN was noted. Bechoua et al. [45] demonstrating that exposure to atmospheric pollution decreases the lymphocyte response to phytohemagglutinin. Furthermore most females in district 1 were housewives constituting the majority of study individuals 56% (57). Housewives had higher NDI when compared to other groups. The possible explanation is that besides difference in exposure to putative causative agents, it is plausible that both genetic and epigenetic effects play roles in these differences. in addition, gender-specific lifestyle and behavioral factors may modulate the effects of exposure to genotoxins [50].Some studies showed positive associations between occupational exposure and genotoxic effect [51,3,52]. However, other studies did not reach the same conclusion [7,53]. Such disagreement may be explained by different exposure conditions. The majority of participants were currently non-smokers. It is interesting that smoking habits did not influence, the cytogenetic parameter examined (BNMN and NDI) **(Table** 5), which is in agreement with a large collaborative study of 30 leading European laboratories [54]. Despite the well-known presence of many genotoxic and cytotoxic in cigarette smoke. There are different results in the literature about the effects of smoking on induction of micronuclei in peripheral blood lymphocytes. Few studies have shown an association between these variables [55,56], while most of the studies of this nature have not found any association at all [57-61]. Bonassi et al. [54] suggest that smoker don't experience an overall increase in MN frequency, although when the interaction with occupational exposure is taken into account heavy smoker were the only group showing a significant increase in genetics damage as measured by micronuclei assay.

Studies evaluating nuclear division or cytokinesis proliferation block index are scarce. Palus et al. [62], using NDI, reported no difference between a smoker group and its control group. A possible explanation is that the damage caused by tobacco could kill the cells in culture or delay the cell cycle, making it impossible to carry out the MN test [54]. However, there was no influence of smoking habit on NDI. Another possibility is that the small number of smokers in the sample study (13 and 5 individuals from district 1 and 2, respectively) constitutes a non-representative sample (**Table 5**).

The present study reports the results of a first estimation the mean count of BNMN in peripheral blood lymphocytes of healthy donors in Iraqi population. This work reveals many background data that may be of value in future genotoxicological monitoring studies in Iraq. Moreover, the results obtained may be used for future comparisons with results obtained on general populations in other cytogenetic laboratories worldwide. The mean count of BNMN in sample study was substantially higher than the reference value for the general population indicating that individuals participating in the present study have been exposed to an action of one or more micronuclei inducing agents that elicit high BNMN mean count. District 1 individuals had higher mean count of BNMN than those district 2 did indicating a significant in vivo genetic damage and cytotoxicity. Reduction of nuclear division index in the study sample particularly in district 2 could be explained by the fact that the population in this district was exposed to an agent that has cytotoxic properties effecting the cell proliferation kinetics and considered as an early marker of cytotoxicity and genotoxicity.

Conflict of interest

The authors declare that they have no conflict of interests.

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