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Research Article

Detection of Micronuclei Formation in Petrol Station Pump Attendants in Awka, Awka South, Anambra State, Nigeria.

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ABSTRACT
The study aimed to detect the extent of micronuclei formation in petrol station pump attendants chronically exposed to petrol fumes in Awka Metropolis, Anambra state, Nigeria.70 participants, made up of 35 exposed and 35 unexposed persons (control group) in the age range of 20-49 years were recruited into the study. Buccal smears were collected from the study participants and used to prepare slides in duplicates for every participant for micronucleus assay. These cells were examined under the microscope after appropriate staining for micronuclei and the frequencies of MN detection were scored per 1000 cells in a field. Analyses of data obtained were done as t-test and Pearson product moment correlations using Minitab 16 software. There was a significant difference in micronuclei detection between the exposed and control groups (p-value= 0.027)). Beyond 2 years of exposure, length of exposure had a weak positive correlation with the number of detected MN (r=0.455). Increasing age and sex did not have any significant effect on the detection of MN in both the exposed and the control groups. The wearing of personal protective equipment (face masks) may be advocated to minimize the quantity of petrol fumes inhaled by the pump attendants during any shift. Self-service pumps could be installed, where feasible, to further reduce the amount of exposure of pump attendants. Pre-employment screening for MN and periodic follow up may be necessary in this category of people. Those attendants with more than normal MN frequency for a healthy population, may be advised to look for alternative employment to reduce their cancer risks from chronic exposure to petrol fumes.

Key words micronucleus, petrol fumes, buccal smears

INTRODUCTION

Cancer prevention is a topical issue all over the world today, because of the ravaging effect cancer morbidity and mortality have on the global population. Statistics have shown that more people die from cancer every year around the world, than from AIDS, tuberculosis and malaria combined[1.] According to[2]. the global burden of cancer had risen to 14.1 million new cases and 8.2 million cancer deaths in 2012 compared with 12.7million and 7.6 million respectively in 2008. One of the strategies for primary prevention of cancer is screening with the help of micronuclei (MN) detection using MN assay of exfoliated cells. Micronuclei are small, extra-nuclear bodies that arise from acentric chromosome fragments or from whole chromosomes that are excluded from the nucleus during mitotic cellular division[3]. MN assay offers a cheap and simple way of screening (bio-monitoring) of a large population for nuclear anomalies which are usually observed in the early stage of the cancer disease process. The presence of nuclear anomalies could also indicate increased risk of developmental and degenerative diseases[4]. The key advantage of the MN assay is the relative ease of scoring and the statistical power obtained from scoring larger number of cells than are typically used for metaphase analysis[5]. Besides the buccal epithelium, the presence of MN can also be evaluated in many other tissues involving dividing cells, for example, cervical epithelium, bladder, oesophagus, and bronchial mucosa. The average reported health population MN frequency is 1-3 per 1000 cells, with intra-individual variability of 30-103%[6]. MN assay as an endpoint of genotoxicity, is a non-invasive and economical procedure with high sensitivity (94%), specificity (100%) and accuracy (95%)[7]. The present study aimed to detect micronuclei in petrol station pump attendants, chronically exposed to petrol fumes, as well as in individuals not exposed to petrol fumes, using MN assay.

MATERIALS AND METHOD

The study population comprised petrol station pump attendants in 25 filling stations in Awka metropolis, Awka South L.G.A., Anambra State.70 participants were recruited into the study. The exposed and control groups were matched by number, sex and age. Each group was made up of 14 males and 21 females in the age ranges of 20-49 years. The exposed group was made up of participants drawn from seven filling stations. Included in the study were persons aged between 20 and 49 years, with exposure period between 6 months and 8 years, non-smokers, with no history of heavy alcohol use, not exposed

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Table 1: Micronuclei detection in both the exposed and control groups

	Exposed	Control
Number of subjects (N)	35	35
MN frequency	14	3
Number of detected MN	60	8
Mean detected MN ±SEM	1.71±0.64	0.23±0.14

Table 2: Relationship between length of exposure and MN detection (N=35)

Duration (in year)	½ ≤ 2	2^+
Proportoin of subjects exposed	30	5
MN ferquency	10	3
Number of detected MN	46	14
Mean detected MN±SEM	1.53±0.68	2.80±2.08

to x-ray less than 6 months before the study, with no history of diseases (apart from malaria) in the preceding 3-4 months and not on any medication. Exclusion Criteria were age less than 20 years or greater than 49 years, absence of exposure to petrol fumes, smoking habit, heavy alcohol consumption, exposure to x-ray less than 6 months before the study, history of disease (apart from malaria) and current history of being on medication.

Materials

The following materials were used for the MN assay: wooden spatula, microscope slides, slide box, giemsa stain (dye), methanol: acetic acid mixture, sachet water, surgical gloves and pencil for labeling the slides.

Sample collection

Samples were collected by asking participants to rinse their mouths properly with water (in order to remove any unwanted debris) before the buccal smears were collected. Sterile disposable wooden spatulae were used to obtain cell samples from the buccal mucosa by scraping the inside of both cheeks. Samples were collected once. The collected samples were air dried and fixed in methanol: acetic acid (3:1). Collected samples were made into two slides for each participant. These slides were sent to the Histopathology Laboratory at Nnamdi Azikiwe University Teaching Hospital (NAUTH) Nnewi at the end of each sampling exercise for further processing and subsequent analysis. The slides were also sent to Histopathology Laboratory, National Hospital Abuja for confirmation. The protocol for the micronucleus (MN) assay was adapted from Fenech et al.[5] and the criteria for defining a micronucleus were according to Tolbert et al.[8].

RESULTS

The effect of exposure to petrol fumes on micronuclei detection

The result shows that 14 cases of MN and a total of 60 MN (mean of 1.71±0.64) were detected in the exposed group. In control group, 3 cases of MN were detected and total of 8 MN (mean 0.23±0.14) (Table 1). Two-sample T – test of the mean difference between the exposed and control was significant, with a p-value of 0.027.

The effect of length of exposure to petrol fumes on micronuclei detection

The result shows that the mean number of micronuclei detected was higher among those attendants who had been exposed for over 2 years (2.80±2.08) compared to those with less than two years of exposure who had a mean MN occurrence of 1.53±0.68 (Table 2). Two-Sample T-Test of means difference between exposure period of less than 2 years and exposure period of more than 2 years was not significant with a p-value of 0.381: Pearson product moment correlation between exposure period of less than 2 years and MN detection gave a correlation coefficient r, -0.058 and a p-value of 0.761. For exposure period of more than 2 years and MN detection, coefficient of correlation, r was 0.455, while the p-value was 0.305.

The effect of age on micronuclei detection in exposure to petrol fumes

From the result, it is seen that the mean number of cases of micronuclei formation detected was highest in the age range of 40-49 years in both the exposed (3.5±2.50) and control (0.50±0.50) groups. In the 20-29 years age range, the mean MN formation in the exposed group was 1.59±0.7, and 0.21±0.15 in the control group. In both the exposed and control groups, no micronuclei were detected in the 30-39 years age range (Table 3). Two-sample T test of means difference between the age range of 20-29 years and 40-49 years was not significant with a p-value of 0.351.

The effect of sex on micronuclei detection in exposure to petrol fumesz

The result shows that in both the exposed and control groups, mean values for micronuclei detection were more in the males than the females. In the exposed group, the mean micronuclei detection for males was 2.07±1.25; whereas in the control group it was 0.29±0.29. On the other hand, the mean values for MN formation in the females were 1.48±0.70 in the exposed group, and 0.19±0.13 in the control group (Table 4). Two-sample T test of means difference between male and female in micronuclei detection was not significant with a p-value of 0.657.

DISCUSSION

The exposed and control groups were made up of 35 study participants in each group, matched for age and sex. In the two groups, participants in the 20-29 years age range constituted 83%, those in the 30-39 years range constituted 5.7%, while the remaining 11.3% was made up by those in the 40-49 years age range. By sex, both the exposed and the control groups were made up of 60% females and 40% males. In the exposed group, 80 per cent had had exposure for less than 2 years (6 months to 2 years), whereas those with exposure duration of more than 2 years made up the remaining 20%. Exposure to petrol vapour is classified by the International Agency for Research on Cancer (IARC) as possibly carcinogenic to humans (IARC class 2B). Studies which demonstrate the genotoxicity of petrol fumes using MN assay include those by[9-16]. Micronuclei detection in individuals chronically exposed to petrol

Table 3: Relationship between age and micronuclei detection

Exposed group (N=35)	Control group (N=35)					
Age (in years)	20-29	30-39	40-49	20-29	30-39	40-49
Subjects per age range	29	2	4	29	2	4
MN frequency	10	0	3	2	0	1
Number of MN detected	46	0	14	6	0	2
Mean detected MN ±SEM	1.59±0.70	0	3.50±2.50	0.21±0.15	0	0.50±0.50

fumes is influenced by such factors as the volume of petrol sold during shifts and the average concentrations of benzene, toluene and the total volatile organic compounds (VOCs) contained in the petrol, age of the attendant, sex, length of exposure, life style (habits such as smoking, and abuse of alcohol), DNA repair capacity of the individual and the levels of antioxidants such as superoxide dismuthase (SOD) and glutathione peroxide (GPx) in the attendant's blood[12]. Factors which could also affect the results of MN assay include differences in timing and implements used in cell collection, fixation, staining techniques, number of cells counted, scoring criteria and other nuclear abnormalities in normal or degenerated cells[4]. Although micronuclei could be detected even in healthy individuals, exposure to petrol fumes which contain various genotoxins, especially benzene is known to cause increased formation of micronuclei in the exposed individuals. The average reported healthy population MN frequency is 1-3 per 1000 cells with intra-individual variability of 30-103%[6]. Findings from this study had revealed a significant difference between the exposed and control groups (p-value was 0.027). Mean MN detected in the exposed group was 1.71±0.64, whereas in the control group it was 0.23±0.14. These results are comparable with what had already been reported by[17]. who found a mean MN frequency of 1.1±0.06 among non-smoker fuel dispensers and 0.06±0.03 among non-exposed non-smokers. However, the present findings contrast sharply with what was reported by a group of Indian researchers in 2003 who found a mean MN frequency of 7.1±3.1 among non-smoker fuel dispensers and 2.1±0.02 among non-smoker non-exposed control[18]. This apparent disparity in micronuclei detection observed between the exposed and the control groups could be largely attributed to the effects of the constituents of petrol fumes as the effects of confounders (exposure to other fumes such as vehicle exhaust fumes, fumes from other petroleum products of various sources, smoke from cigarettes as in passive smoking among others) had been reduced to the barest minimum by recruiting the control group from a rural locality where the effects of these confounders (air pollution from exhaust fumes of vehicles and fumes from other petroleum products) were minimal. From toxicological principles (dose-response relationship), the length of exposure to petrol fumes would be expected to correlate positively with the frequency of MN detection. In this study that was not the case. Although findings from the study revealed that mean MN occurrence was higher in those exposed individuals with duration of exposure more than 2 years (3.50±2.50) than in those exposed for less than 2 years (1.61±0.73), statistically there was no significant difference in MN detection between these two periods of exposure (p-value =0.381). Here the statistical inference did not follow the toxicological principle of dose-response relationship in which the response to the effect of a given toxic substance usually increases as the dose and duration of exposure to the substance increase. Medically too, the statistical inference did not hold, since the amount of damage done to the cells in the exposed individuals would be expected to increase as the dose and length of exposure to the toxic substance increased. For those individuals exposed for less than two years, there was a very weak negative (negligible) correlation between duration of exposure and MN detection (r= -0.058, p-value = 0.761); whereas for those with duration of exposure more than two years, there was a moderate positive correlation between duration of exposure and MN detection (r= 0.455, p-value = 0.305)[12,16,19-22]. were all able to demonstrate a positive correlation between length of exposure and the degree of MN detection in individuals chronically exposed to petrol fumes. However[13,23]. could not establish such a correlation. The finding of a relatively higher mean MN (3.5±2.50) in individuals with length of exposure greater than 2 years compared with those exposed for less than 2 years (1.61±0.73) could be attributed to greater genotoxic effect of increasing volume of dispensed petrol as the duration of exposure increased. This means greater exposure to the genotoxins contained in the petrol fumes, hence the greater the number of MN detected as a biomarker of this insult. A relatively lower number of MN detected in those individuals with exposure less than two years could be that in addition to less volume of petrol dispensed by them, compared to the volume dispensed by those with exposure beyond two years, at the beginning of the insult (the action of petrol fumes), the body still had some reserve of the compensatory mechanisms to battle with the effects of the genotoxins. However, as the length of exposure increased, these compensatory mechanisms could begin to fail as a result of greater accumulation of genotoxins contained in the fumes, hence the increasing number of MN detected in these individuals[12]. Another major factor that could affect MN formation due to the effect of petrol fumes in the exposed individual is the age. Generally, aging affects the rate of cells turnover, and consequently the rate of DNA damage and repair. As a matter of fact, as we age, our cell renewal factor or cell turnover rate slows down and there is accumulation of DNA damage[24]. The study revealed a higher mean number of detected MN as the age of the exposed individual increased (3.50±2.50) in those aged between 40-49 years, compared to 1.52± 0.70 in the age range of 20-29 years. This difference was also noted in the control group (0.50± 0.50 for age range 40-49 years and 0.21± 0.15 for age 20-29 years). However, there was no statistical difference between age 40-49 and 20-29 in mean

Table 4: Relationship between sex and micronuclei detection

	Exposed group (N=35)		Control group (N=35)	
Sex	M	F	M	F
Proportion of subject	14	21	14	21
MN frequency	3	10	1	2
Number of detected MN	29	31	4	4
Mean detected MN±SEM	2.07±1.20	1.48±0.70	0.29±0.29	0.19±0.13

M N detection (p-value = 0.351). Opinion about the effect of age as one of the factors that affect MN detection in individuals chronically exposed to petrol fumes is divided. While some studies report a worsening DNA damage and MN formation from chronic exposure to petrol fumes as age increases, others report no correlation[12,16,19-21]. reported a positive correlation between increasing age of the exposed individuals and the number of MN detected in these individuals[25]. also found a positive correlation between increasing age and MN detection, but this was in a healthy population study. On the other hand[9,13,26]. could not find any correlation between increasing age and the frequency of MN detection in individuals chronically exposed to petrol fumes, implying that increasing age of the individual does not affect the degree of MN formation in chronic exposure to petrol fumes. The finding of a higher mean MN detection in the age range of 40-49 years (3.50± 2.50) compared to 1.59± 0.70 in the range of 20-29 years could not be solely attributed to the effect of the petrol fumes since this tendency was also noticed in the control group. As suggested by[25]. in a study of a healthy Japanese population, the observed increase in MN frequency due to age could be attributed to possible accumulated genetic damage occurring with increasing age. Assuming this to be so, in the present study it appears then that exposure to petrol fumes aggravated this age dependent process, hence the observed higher mean detected MN in the exposed, compared to the control group. Finally, another factor that could affect MN detection in individuals with chronic exposure to petrol fumes is the sex of the exposed individual. Because of the differences in the male and female physiology, these two sexes could differ in the degree of MN detection from chronic exposure to petrol fumes. The sex chromosome is lost 22 per cent more often in females than in males because female's cells are more sensitive to the action of genotoxic agents[27]. This could be the reason some studies had reported higher frequencies of MN detection in female subjects chronically exposed to petrol fumes, compared to their male counterparts. Findings from the study had demonstrated a slightly higher mean MN detection in males (2.00±1.30) than females (1.48±0.70). This difference was also noticed in the control group, where the mean MN detected in males was 0.29±0.29, whereas in females it was 0.19±0.13. These findings were in agreement with the findings of[11,23,28]. who also demonstrated higher MN frequencies in the males exposed chronically to petrol fumes, compared to the females[25]. similarly reported a higher MN frequency among males when compared to females, but in a healthy population. Unlike the studies demonstrating higher frequencies of MN detection in males of the exposed group[22] in a recent study found a higher frequency of MN detection in female subjects of both the exposed and control groups. On the other hand[29]. did not find any relationship between sex and the degree of DNA damage. Although there was a slightly higher mean detected MN in the males of the exposed group (as also in the control groups) than in females, there was no statistical difference between both sexes in the exposed group in the detection of MN (p-value was 0.657). Higher frequency of MN detection in male subjects as reported by some researchers had been speculated by[25] to be probably due to the fragility of the Y-chromosomes when compared to other chromosomes or due to unknown factors. In the present study, such factors also seem to have been at play, since slightly higher frequency of MN detection was seen in males of both the exposed and the control groups. The observed slight increase in the frequency of micronuclei detection in the males of both the exposed and the control groups, compared to the females could also reflect differences in the physiology of the male and female bodies or impaired mechanisms of DNA damage repair resulting from compromised body homeostasis[30].

CONCLUSION AND RECOMMENDATIONS

In conclusion, the study of the effects of chronic exposure and some demographic factors on the detection of micronuclei as a biomarker of genotoxicity of petrol fumes in petrol station pump attendants in Awka Metropolis, Anambra state, Nigeria was able to establish a significant difference between individuals chronically exposed and those not exposed to the fumes. There was a moderate positive correlation (*r=0.455*) between length of exposure to petrol fumes and micronuclei detection. Age and sex of the individuals occupationally exposed to petrol fumes did not have any correlation with the frequency of detection of micronuclei in these individuals. In view of these findings, it may then be recommended that face masks be regularly worn by the pump attendants on duty to minimize the amount of petrol fumes inhaled by them on any shift. Self-service petrol stations (where feasible) could be established to further minimize the period of contact of the pump attendants with petrol fumes. Periodic screening for the genotoxicity end points such as micronuclei formation using MN assay may be necessary to assess the degree of risk of cancer development from inhalation of petrol fumes. It may be appropriate to do MN assay on the petrol station pump attendants at the point of entry into the employment (as a component of pre-employment screening) and at predetermined intervals to see the trend in MN formation as age and duration of exposure to petrol fumes increase. Those attendants with increasing frequencies of MN detection that have exceeded the

normal for a healthy population (1-3 per 1000 cells) may be advised to look for alternative employments to prevent further increase in the risk of cancer development attributable to exposure to petrol fumes.

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