Monitoring of Human Populations at Risk by Different Cytogenetic End Points

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Humans are exposed to a large number of environmental genotoxic agents. These can increase the probability that somatic mutation will occur. The use of genotoxicity testing is essential for assessment of potential human toxicity so that hazards can be prevented. Cytogenetic monitoring of human populations exposed to chemicals has proved to be a useful tool for detecting the chemical mutagenic effects. Cytogenetic analysis of human chromosomes in peripheral lymphocytes allows direct detection of mutation in somatic cells. Cytogenetic monitoring of a group of traffic policemen from Cairo, Egypt, was an example of a human population study. The induction of chromosomal damage was studied in a group of 28 traffic policemen with exposure of over 10 years and a control group of 15 policemen trainers. Blood lead level was significantly higher in the traffic policemen (30 ± 8.7) unit compared to the control group (18.2 ± 1.2) unit. The percentage of chromosomal aberrations (7.7 ± 3.1), as well as the mean sister chromatid exchanges (7.5 ± 3.4), were significantly higher among the traffic policemen than in the control group. The percentage of chromatomal aberrations was 2.8 ± 2.1 and the mean sister chromatid exchanges was 4.8 ± 2.9 in the control group. On the other hand, the increase in chromosome damage among the traffic policemen was enhanced further by smoking. Several problems that are found in biomonitoring studies are discussed. — Environ Health Perspect 102(Suppl 4):131–134 (1994).

Key words: chromosome aberrations, SCE, lymphocytes, traffic, policemen, human monitoring

Introduction

Humans are exposed to a large number of physical or chemical genotoxic agents. Exposure to these agents can cause a variety of health hazards. Some of them are expressed immediately; others take years. The later type of abnormalities include the induction of cancer or genetic diseases. The exposure patterns are complicated with respect to exposure to single agent or complex mixtures. These agents may act synergistically or with inhibitory effects. Therefore, continuing efforts are being made to identify hazardous agents, to recognize hazards such exposure conditions, and to monitor populations for signs of excessive exposure to prevent adverse health consequences in the population.

It has been shown in experimental systems that many environmental agents can be mutagenic or carcinogenic or both. Identification of mechanisms of cancer development has involved consideration of the somatic mutation hypothesis, on the basis of the widespread occurrence of chromosomal abnormalities in cancer cells.

Subsequent correlations between the mutagenicity and the carcinogenicity of radiation and chemicals have provided considerable support for this hypothesis (1).

Recently, increasing attention has been paid to the development of monitoring methods by which human exposure to mutagens and carcinogens can be detected. Several biomarkers were developed for this purpose (2). Epidemiologic studies on cancer development in humans are necessary for risk assessment approaches. However, the epidemiologic approach is limited for two main reasons: first, only relatively high risks can be detected, and second, the observations on end-effects are the consequence of exposures that may have occurred several decades earlier. Improved epidemiology, ideally, needs direct and accurate estimates of individual exposures. Biomonitoring has become an essential part in the exposure assessment, its special objective defining biologically relevant doses. It may be relevant to look for early effects directly in the exposed individuals or groups, especially from high exposure occupations. Presently, there is a need for multidisciplinary studies to evaluate the effect of different genotoxicants.

The recommended methodology for risk assessment of environmental genotoxicants is to determine the external exposure by environmental monitoring, the internal absorption by biological monitoring, and the biological effect by genetic monitoring. In addition, clinical response can be determined by epidemiologic studies (3). Proper and relevant methods for genotoxicity assessment need to be used both at the experimental (4,5) and at the human exposure assessment levels (6).

Egypt has a large population of about 60 million. Out of this population, about 12 million people are living in Cairo. There is a high number of automobiles that contribute to air pollution. In recent years, the incidence of cancer in Egypt has increased. Lung cancer, which is mostly related to air pollution, is more common in urban cities in comparison to rural villages. In urban areas, exposures to low levels and short-term peak levels of engine exhausts are ubiquitous (7). Higher exposures to engine exhausts may occur in some occupations such as traffic policemen.

Many studies have been carried out, using several animal species, to evaluate the potential carcinogenicity of exposure to whole exhaust and to components of exhaust from diesel- and gasoline-fueled internal combustion engines (8–11). The genetic and related effects of diesel and gasoline engine exhausts have been reviewed (9,12–15). The presence of mutagens and carcinogens can be detected in the ambient air by the use of the Salmonella (Ames) assay system (16,17). Using this short-term assay, pollutants from automobile exhaust have been shown to contain direct and indirect-acting muta-
gens (18-21). Solvent extracts of airborne particulates from many locations contain compounds that induced sister chromatid exchanges and chromosomal aberrations in cultured human lymphocytes (22).

In 1972, Bauchinger et al. (23) studied the cytogenetic effect of automobile exhaust on human peripheral blood lymphocytes in 29 policemen. They found no significant increase in chromosomal aberrations. Because mutagens have been found in automobile exhaust, its genotoxic effect in exposed humans should be adequately evaluated. It is perhaps relevant to study individuals with more extensive exposure to the exhaust.

Material and Methods
Population cytogenetic monitoring is one of the ways in which the effects of environmental mutagens can be detected efficiently in man. A small peripheral blood sample provides enough cells to be scored for chromosomal aberrations. Blood collections are technically easy, and it is therefore possible to carry out repeated periodical sampling of exposed populations.

A study was carried out to evaluate the cytogenetic effects in humans exposed to automobile exhaust in Cairo, Egypt (24). Cairo is an overcrowded nonindustrial city of approximately 12 million people. Heavy traffic exists on all major roads where traffic policemen are on duty to maintain the flow of stop and go traffic. The policeman are on duty for 8-hr/day for 6 days/week. Because it seldom rains in Cairo and because most cars are not well tuned, the exposure to car exhaust could be at a very high level for these policemen. The induction of chromosomal damage was studied in a group of 28 traffic policemen with exposure of over 10 years and a control group of 15 policemen trainers. Blood lead level was determined in the traffic policemen as well as in the control group using atomic absorption spectrophotometer (Perkin Elmer model 400) supplied with graphite furnace (Perkin Elmer model HG 400). Analysis of blood lead level has been shown to be a good indicator for exposure to automobile exhaust (25). The two cytogenic end points used in this study follow.

Conventional or Classic Cytogenetic Technique
Giemsa staining of chromosomes was the first classic cytogenetic technique for testing the mutagenic effects on human chromosomes. This technique permits rapid overall analysis of tested cells (i.e., checking of the chromosomal number and registration of chromosomal damage) (26). The chromosome aberration assay proved to be one of the most sensitive and relevant tests for identification of mutagens and carcinogens (27). Although it is not as specific as molecular studies of effects on single genes, the assay monitors the entire genome and proved to be a useful population-monitoring procedure. Exposure to hazardous agents can cause both chromosome breakage and rearrangement (28).

Harlequin Technique
Sister chromatid exchanges (SCEs) are cytologic manifestations of DNA double-strand breakage and rejoicing at homologous sites between two chromatids of a chromosome. These exchanges can be detected easily by growing lymphocytes in a medium containing thymidine and 5-bromodeoxyuridine for two cell cycles followed by autoradiography or differential staining, respectively. This technique has been extensively used in human population monitoring. It has been described as a sensitive, convenient method for monitoring exposure to environmental genotoxic agents. The detection of SCE in cells is more rapid than scoring of the classic aberrations. SCEs occur by different mechanism than the classic aberrations; therefore, we are probably testing two different types of DNA damage. For these reasons, the harlequin technique is convenient for large screening studies. It is generally observed that among the lymphocytes, there is a small population that contain high frequencies of SCEs. These high frequency cells (HFCs) can be found both in controls and exposed populations. Comparison of frequencies of SCEs in HFCs has been shown to be very sensitive in detecting exposure to chemicals (29,30).

| Table 1. Frequency of chromosomal aberrations and sister chromatid exchanges (SCEs) in a group of traffic policemen and in the control group (smokers and non-smokers). |
|---------------------------------------------------------------|
| % of cells with chromosomal aberrations | SCEs/cell, mean ± SD |
|-----------------------------------------------|---------------------|
| **Control group** |                      |                    |
| Smokers      | 3.4 ± 2.5 (n = 7)   | 5.0 ± 2.8          |
| Nonsmokers   | 2.3 ± 1.8 (n = 8)   | 4.7 ± 2.4          |
| Combined     | 2.8 ± 2.1 (n = 15)  | 4.8 ± 2.9          |
| **Traffic policemen** |               |                    |
| Smokers      | 8.5 ± 2.9 (n = 22)  | 7.6 ± 3.4          |
| Nonsmokers   | 4.5 ± 1.4 (n = 6)   | 7.1 ± 3.4          |
| Combined     | 7.7 ± 3.1 (n = 28)  | 7.5 ± 3.4          |

*100 metaphases were analyzed per person for the presence of chromosomal aberrations. *b Significantly different from nonsmokers of the same group (p<0.05). *c Significantly different from the combined responses of the control group (p<0.01).

Results
There was no significant difference between the exposed and the control group concerning age. As shown in Table 1, the percentage of chromosomal aberrations (7.7 ± 3.1) as well as the mean SCEs (7.5 ± 3.4) were significantly higher among the traffic policemen than in the control group. The percentage of chromosomal aberrations was 2.8 ± 2.1 and the mean SCEs was 4.8 ± 2.9 in the control group. The increase in chromosomal damage among the traffic policemen was enhanced further by smoking. The difference between smokers and non-smokers was statistically significant in case of chromosomal aberrations and not significant in case of SCEs. The detected chromosomal aberrations were mainly gaps and breaks. Dicentrics, fragments, and exchanges were rarely detected. A small number (0.4%) of cells containing polyplid chromosomes also was observed in the traffic policemen. The highest frequency of SCEs (more than 10) were detected in traffic policemen who were smokers. The lowest frequency (less than 6) were found mainly in the control non-smoker group. The increase in chromosomal damage was not correlated with the blood lead level or the duration of employment.

Discussion
The results of the present study indicate that traffic policemen have significantly higher frequencies of chromosomal aberrations and SCEs than the controls. These findings are different from that of Bauchinger et al. (23), who reported no increase in aberrations. The discrepancies can be explained by different design of the studies. In the present study, the exposed group has a minimum exposure time of 10 years, which is substantially longer than the group studied by Bauchinger et al. Moreover, the blood lead level showed a 67% increase in
the traffic policemen in the present study compared to the controls. On the other hand, it was 20 to 30% increase in the other study. Although Bauchinger et al. did not find significant differences between the exposed and the control groups, some types of aberrations (dicentrics and chromatid exchanges) were found only in their exposed group. In addition, mitotic frequencies also were reduced compared to the control group.

Smoking increased the chromosome aberration frequencies more among traffic policemen than in the control group (p < 0.05). Therefore, we suspect that there is an interaction between smoking and exposure to automobile exhaust that produce synergistic effect in induction of chromosomal aberrations.

Evidence suggests that chromosomal changes may be intrinsically linked to cancer development. Chromosomal instability is characteristic of dysplasias and many premalignant conditions, and specific chromosomal aberrations appear to be associated with many types of cancer (31). These abnormalities can activate oncogenes or result in the loss of tumor suppressor genes (32,33).

An evaluation of chromosomal changes detected in the cells of exposed workers requires reliable control data. It is more convenient to use the cells from the same person before exposure as control, and then, if possible, at various time intervals during and after exposure to the chemical tested (34). This will avoid the difficulties encountered with differing sensitivities of individuals tested. Such controls are optimal but they are seldom available when occupationally exposed persons are tested. Therefore, blood samples from other persons must be used as controls. If possible, the control should be matched in terms of sex, age, and race. Control blood samples should be cultured under exactly the same conditions as samples from exposed people and slides should be coded before scoring. The average percentage of aberrant cells in peripheral blood lymphocytes of healthy adult people, who are not exposed to unusual doses of mutagens, is 1% to 2% in most laboratories, if gaps are not included. Young children and infants usually have less than 1% of aberrant cells (6).

A number of factors, such as the use of drugs, drinking alcohol, smoking habits, radiation, and viral or other infections in the last 3 months before sampling, need to be taken into account because they can have a profound influence on the results. Individuals exposed to elevated levels of radiation and chemicals in the remote past are not suitable as controls because some types of aberrations may survive for years in the human body (7). Intraindividual variability may be because of errors in the laboratory assay, dietary influences, or other exposure conditions. Repeated measurement over time will give a more precise estimate of long-term exposure status.

Chromosomal aberrations are at present only a qualitative indicator of mutagenic effects. If chromosomal aberrations have increased, exposure must be reduced because these individuals also may have other types of genetic damage (e.g., point mutation or chromosomal aberrations in germinal cells). Results of some studies (35,36) have demonstrated that chromosomal changes are reversible, and that chromosomal aberrations may revert to control levels in 2 to 3 years after reduction of the exposure to the mutagen. An additional problem in cytogenetic surveys of populations exposed environmentally is to obtain statistically significant results with regard to low-level exposures. Thus, although chromosomal changes are an indicator of cellular genetic damage in a population, they cannot be used quantitatively to predict future health effects for a given individual (37). In a recent survey of over 100 human cytogenetic studies, conducted between 1965 and 1984, Ashby and Richardson (38) concluded that there is an urgent need for standardization of both study protocols and methods for reporting chromosomal aberrations.

Data from well-conducted cytogenetic studies can be used with confidence to indicate potential risk for development of health problems in the exposed population. However, cytogenetic data alone cannot document risk on an individual basis (28). Cytogenetic data can be combined with other information for estimation of potential health problems. Other useful information include documentation of the presence of active mutagenic and carcinogenic metabolites, biologically relevant DNA and protein adducts, abnormal sperm, and increased frequencies of gene mutations as well as the expression of clinically diagnosed toxicity. The future role of cytogenetic assays in population monitoring will depend on our ability to continue to improve the assay and to conduct well-planned studies.

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