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Cytogenetic Surveillance of Workers Exposed to Genotoxic Chemicals: Preliminary Experiences From a Prospective Cancer Study in a Cytogenetic Cohort

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Cytogenetic endpoints, conventionally chromosomal aberrations. and later sister chromatid exchanges and micronuclei have long been used to assess exposure of human populations to genotoxic agents. Although the adverse nature of somatic chromosome damage is recognized at the p u p level. no ill-health manifestations have been causally related to cytogenetic damage at the individual level. In work-related exposures. e.g., ethylene oxide. styrene, benzene, vinyl chloride. and alkylating anticancer agents have been shown *to* induce somatic chromosomal darnage in several studies. For all of these, a carcinogenic risk to humans has also been documented.

The possible association of somatic chromosome damage and cancer will be elucidated in a Nordic prospective study. The objective is to find out the significance of a high or low score in any of the cytogenetic parametres *to* risk of cancer. In the Finnish part of the cohort of 806 individuals. 10 casesof cancer were observed during the first follow-up period. Although the cohort is young and the numbers small, a slightly significant ($P = 0.04$)trend was observed for individuals with cancer and a score of chromosomal aberrations. No trend was observed for sister chromatid exchanges.

The application of cytogenetic surveillance is still not routine methodology, but it is useful and informative in carefully controlled study designs. Special efforts should be directed toward combining different disciplines, i.e., cytogenetics. adduct monitoring, and end-effect epidemiology. in order to reach quantitativeness in risk assessment,

Key words: chromosomal aberrations, sister chromatid exchanges, population biornonitoring, cancer risk, genotoxic exposure

INTRODUCTION

Human biomonitonng, as a tool to identify and potentially quantify the risk of hazardous exposures, has gained increasing attention especially in the area of cancer

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risk assessment. The novel approaches have been in adduct monitoring, i.e., in measuring quantitatively the amount of genotoxic chemical binding to DNA or other macromolecules, usually in nontarget tissue, as regards cancer outcome. The sensitivity of the physicochemical or immunochemical assays is high, but as yet the chemical lability and kinetics of repair of adducts in DNA are poorly understood, and the relationship of various confounding background exposures to protein adducts has not yet been critically assessed [1].

The more traditional biomonitoring tools are cytogenetic, i.e. measuring microscopically visible chromosome damage in human somatic cells, usually peripheral blood lymphocytes. Similar to adduct studies, the conceptual basis for the application of cytogenetics is in principle that the extent of genetic damage in the nontarget tissue reflects the events in the cells relevant to the carcinogenic process. Concerning somatic chromosome damage, the causality to cancer etiology is based on their association in hereditary chromosome instability syndromes and in some constitutional abnormalities of the karyotype [2]. Chromosomal rearrangements also play an important role in the activation of proto-oncogenes [3]; thus, their association to cancer is indirectly supported, In the following, the possible predictiveness of cytogenetic damage to cancer outcome is elucidated on the basis of a large cohort of cytogenetically studied subjects.

CHROMOSOMAL ABERRATIONS

Chromosomal aberrations consist of overt breakage and rearrangements of chromosomes visualized in the metaphase plate. Chromosomal aberrations are most sensitive to agents that can directly break the DNA duplex, such as ionizing radiation and a few radiomimetic chemicals. Since most chemical mutagens that cause chromatid-type damage are S-phasedependent, the highest yield of aberrations is likely to be seen in the metaphases following first division. Thus, in cytogenetic monitoring only the first division metaphases should be used for scoring. The various technical and study design confounders and their control efforts have been discussed in several earlier reports [4,5].

The cohort collected from subjects cytogenetically studied during 1978–1986 for chromosomal aberrations at the Institute of Occupational Health in Helsinki comprised 476 persons, 454 adults and 22 children.

The means of aberration scoring results in different subgroups are given in Table I. The differencies between the subgroups are small; a well-known insensitivity problem in human exposure studies using chromosomal aberrations as endpoint. The analysis of variance, considering known and potential confounders for chromosomal aberrations, showed age and number of cigarettes smoked daily to be significantly ($P < 0.001$), associated with chromosomal aberrations (gaps excluded), whereas no effect of sex or specific exposure status was observed. When the material was trichotomized for later cancer outcome studies, the percentile limits for low (0-33 percentile), medium (34-66 percentile), and high 67–100 percentile) were at 1.0% and 3% (total aberrant cells excl. gaps) (Fig. 1).

The general criteria for inclusion into the study cohort and the references to the original publications describing the subgroups studied have been given in the description of the collaborative Nordic Study Cohort [6].

SISTER CHROMATID EXCHANGES

Sister chromatid exchanges involve breakage of double-stranded DNA in both chromatids followed by an exchange of whole DNA duplexes. These events occur in the

TABLE I. Pooled Scoring Results of Chromosomal Aberrations From One Laboratory: 1978–1986

Group	No. of persons	No. of cells with aberrations (excl. gaps) Mean	SD
All adults	454	2.53	2.22
Males	311	2.40	2.03
Females	143	2.80	2.59
Children	55	2.01	2.27
Smokers	222	2.80	2.45
Nonsmokers	ex. 40	2.72	2.72
	never 192	2.17	1.72
Exposed	268	2.58	2.38
Controls	186	2.45	1.99

S-phase, and SCEs are thus most efficiently induced by chemicals that form covalent adducts to the **DNA,** distort the **DNA** helix, or interfere with **DNA** precursor metabolism or repair. The exact molecular mechanism of SCE formation is still unknown, but **SCEs** are not considered to be mutational events.

The method for preparation of metaphases for sister chromatid exchange analysis is analogous to that for chromosome aberrations, except that 5-bromodeoxyuridine (BudR) is added to the cell culture medium and incorporated for two rounds of replication, enabling visualization of the exchanges through differential staining.

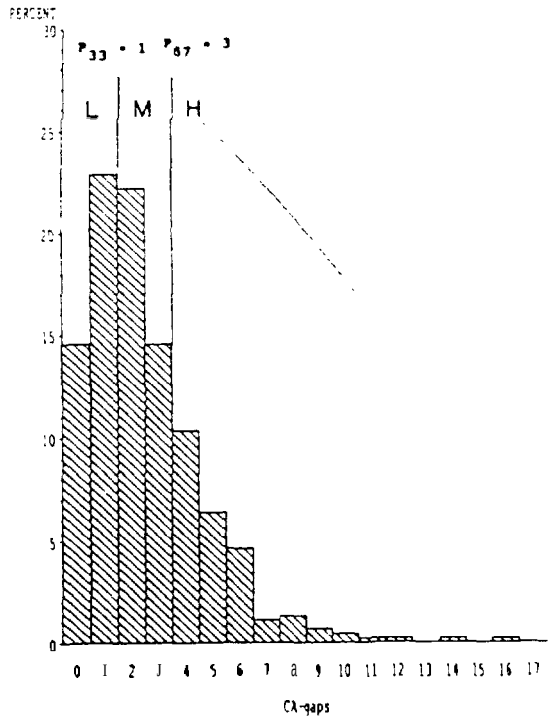

Fig. 1. Distribution of individual values of chromosomal aberrations (% aberrant cells. gaps excluded) in the cohon of 454 adult subjects studied in one laboratory. The percentile limits are indicated to divide the subjects into low (L), medium (M), and high (H) categories of scoring result.

TABLE II. Pooled Scoring Results of Sister Chromatid Exchanges From One Laboratory: 1978–1986

Group	No. of persons	Sister chromatid exchanges	
		Mean	SD
All adults	656	8.89	1.97
Males	366	9.30	2.21
Females	290	8.37	1.46
Children	23	5.11	1.09
Smokers	288	9.71	2.15
Nonsmokers	ex. 60	8.27	1.43
	never 308	8.24	1.56
Exposed	330	9.19	2.26
Controls	326	8.59	1.56

The study cohort of subjects studied for sister chromatid exchange frequency at the Institute of Occupational Health in Helsinki during 1978–1986 comprises 679 persons, 656 adults and 23 children (Table II). The analysis of variance showed age to be significantly ($P < 0.001$) associated with SCE (children were excluded from this analysis). The low SCE frequency of children has been noted also in other studies [7,8]. The number of cigarettes smoked daily was significantly ($P < 0.001$) associated with SCE frequency; a fact also well documented in earlier studies (see IARC 1986). Other factors significantly associated with mean SCE were sex ($P < 0.01$) and being "exposed" as compared to being a "control" person in the study ($P < 0.001$). The former result may be affected by the fact that males are usually heavier smokers. The "exposed" group consists of subjects studied because of a specific occupational chemical exposure. The largest "exposed" groups included are workers in the rubber industry, nurses and workers handling anticancer agents, and persons exposed to tobacco smoke (see 6 for references).

In the plotting of the individual mean SCE analysis data. the percentile limits dividing the subjects into the low (= L), medium (= M) and high (= H) SCE rate category were $P_{33} = 7.8$ and $P_{67} = 9.3$ (Fig. 2). These limits were used for the linkage in cancer outcome.

CYTOGENETIC ENDPOINTS AND CANCER OUTCOME

It is reasonable to expect that the usually less than 7–8% values of chromosomally damaged lymphocytes have no significance as such to the health of the individual. With higher values, normally not observed in occupational chemical exposure situations, immunosuppression is a possibility, as seen in high dose radiation damage, which manifests as cell death and acute radiation illness. With radiation-induced chromosome damage, the kinetics of reduction of chromosome damage, in response to replacement of the lymphocytes, is well documented [9], whereas there are only a few examples of chemical exposures [5]. The main relevance of lymphocyte chromosome damage is in its indicative value of genotoxic exposure, which has an indirect impact on carcinogenesis and possible other pathological conditions relating to focal lesions [10].

At the group level, exposure to carcinogenic genotoxic chemicals and induced cytogenetic damage correlates with cancer risk (see Sorsa et al. [11] and references cited therein). In an experimental study with rats exposed to three doses of ethylnitrosourea, no individual correlation was found in the SCE response and tumor occurrence, although this was seen between the low dose and high dose groups [12].

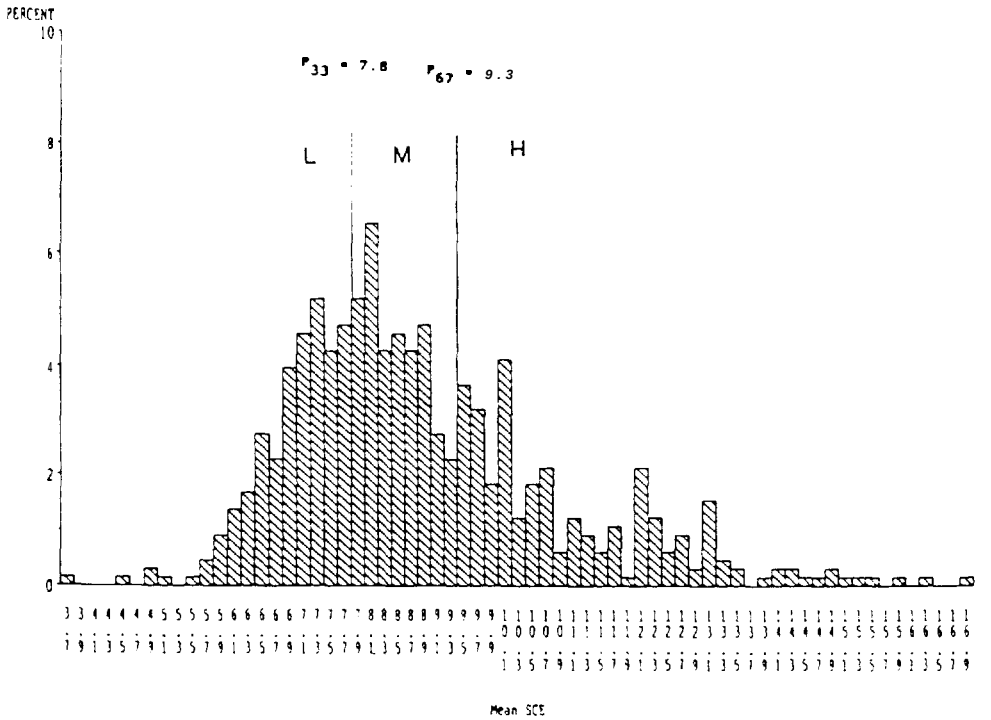

Fig 2 Distnbution of individual mean values of sister chromatid exchange frequencies in the cohort of 656 adult subjects studied in one laboratory The percentile limits are indicated to divide the subjects into low (L), medium (M), and high (H) categories of sconng result

The prospective study on somatic chromosome damage and possible cancer risk at the individual level is a Nordic collaborative effort where altogether ten laboratories from four Nordic countries are taking part. The first collaborative results have been published elsewhere [13]. The data base from Finland comprises 806 adult individuals studied by two cytogenetic laboratories during 1974–1988 either for sister chromatid exchanges **(SCE)** or for structural chromosomal aberrations, or both.

In the cohort, through register linkage to the Finnish Cancer Registry, altogether ten cases of cancer were found during the observation period (1973–1985). The mean latency time between the earlier cytogenetic analysis and the diagnosis of cancer was 4.9 years.

Among the 10 cancer cases observed so far from the Finnish cohort, 9 had been analyzed for chromosome aberrations and 7 for sister chromatid exchanges (Table III). A statistically significant positive trend for cancer risk and chromosome aberrations was seen, whereas no such trend was observed for sister chromatid exchanges. Because the numbers are small and the person years of follow-up still very low, chance cannot be excluded as an explanation for the association. However, power calculations performed for the total Nordic cohort comprising of 3,190 subjects indicate that doubling of the cancer risk should be demonstrable in allowing 5 years more follow-up time (cancer morbidity until the end of 1990) [13].

TABLE III. Relationship Between Frequency of Chromosomal Aberrations and Mean Sister Chromatid Exchange Frequency[a,b]

Percentile category	Obs	Exp	SMR	95% CI
Chromosomal aberrations				
LOW	1	1.2	83	2– 464
Medium	3	1.1	272	56– 797
High	5	1.1	455	148–1061
All	9	3.4	265*	121– 502
Sister-chromatid exchanges				
LOW	2	0.7	286	35–1032
Medium	2	1.0	200	24– 722
High	3	1.7	176	31– 516
All	7	3.4	206†	83– 424

[a]Low, 1–33 percentile; medium, 34–67 percentile: high. 68–100 percentile in peripheral lymphocytes and subsequent risk of total cancer morbidity in the Finnish cohort. Obs, observed number of cancers: Exp, expected number of cancers (national statistics); SMR, standardized morbidity ratio.
[b]Calculations were based on 433 subjects and 2,227 person-years for chromosomal aberration scores and for 483 subjects and 2,335 person-years for sister chromatid exchange scores.
*P-value for trend in SMR = 0.042 (one-tailed test).
†P-value for trend in SMR = 0.3 (one-tailed test).

CONCLUSIONS

Increased frequencies of chromosomal damage can serve as an indicator of exposure to genotoxic agents and signal the potential of cancer risk at the group level. Cytogenetic surveillance of people exposed to ionizing radiation has been carried out for many years, but the methods have yielded positive results only for a limited number of chemical clastogens. Most of the experience has been obtained with agents such as benzene, ethylene oxide, vinyl chloride, styrene, epichlorohydrin, and some alkylating anticancer drugs. The extent of cytogenetic damage is a function of the exposure level, although the dose–effect relationships are rarely obtained in chemical exposure studies. Positive results in cytogenetic surveillance should prompt implementation of hygienic controls or medical surveillance, even in the absence of direct evidence relating chromosomal damage to adverse health outcomes. Prospective studies similar to the one described here should be started to find out such possible relationships.

The available cytogenetic test systems are still too insensitive and tedious for use as a routine surveillance procedure; more effort should be placed on combining cytogenetic surveillance, for example, to adduct monitoring in the same groups of subjects Application of human biomonitoring needs to be done with care and confidence, considering the possible confounding factors and with prerequisite knowledge of the exposing agents in experimental systems. Consequently, the methods are useful and informative under carefully selected conditions. Positive findings indicate that preventive health measures must be taken.

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