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Chromosomal Aberrations in Males Occupationally Exposed to Chemical Pollutants in the Gaza Strip-Palestine

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Abstract

Objective*:* This study was conducted to evaluate the frequency of chromosomal aberrations in peripheral blood lymphocytes from Palestinian males exposed to various chemical pollutants during their daily work.

Subjects and Methods: The study population consisted of 32 males (mean age 35.5 years) distributed as: 14 farmers, 3 plumbers, 5 taxi drivers, 6 paint factory workers, and 4 gas station workers, exposed to pollutants like pesticide, petrol derivatives etc. The control group consisted of 10 healthy Palestinian individuals of the same age and gender, but not exposed directly to pollutants in their jobs. Chromosomes were prepared from peripheral blood lymphocyte cultures using standard methods. The evaluation of chromosomal aberrations was performed following the IPCS (International Program Chemical Safety) guidelines for the monitoring of genotoxicity effects of carcinogens in humans.

Results: A significantly higher incidence (2.14%, $p<0.05$) of chromosomal aberrations (chromatid breaks, iso-chromatid breaks, chromatid deletions, and acentric fragments) were detected in lymphocyte of the study population. Interestingly, no chromosomal damage at all was recorded in the control group lymphocyte.

Conclusion: These results suggest that occupational exposure to chemical pollutants is the cause of the chromosomal aberrations observed in the study population, which could be related to exposure time, since chromosomal aberrations were more frequent in workers exposed for longer times. The increased chromosomal damage detected in the study population can be attributed to the complex mixture of genotoxic compounds to which this group has been exposed.

Key Words: Pollutants, Chromosomal aberrations, Genotoxic, Occupational exposure.

التشوهات الكروموسومية في الذكور المعرضين مهنيا للملوثات الكيميائية في قطاع غزة - فلسطين

الملخص: تهدف هذه الدراسة إلى دراسة تأثير الملوثات البيئية على كروموسومات الإنسان، حيث تم جمع عينات من 32 شخص (ذكور وبمتوسط عمر 35.5 سنة) وكان توزيعهم كالتالي: 14 مزارع، 3 سمكري ، 5 سائق أجرة، 6 عاملون دهان، 4 يعملون في محطة الوقود. والمجموعة الضابطة هم 10 أشخاص أصحاء لهم نفس الجنس ومتوسط العمر ولا يتعرضوا لهذه الملوثات في مجال عملهم.

تم تحضير مزارع الخلايا اللمفاوية وتحضير الكروموسومات وصبغها بالطرق المتبعة عالميا، ومن ثم تقييم الخلل في الكروموسومات بناء على برنامج الأمان الكيميائي العالمي IPCS (International Program Chemical Safety).

أظهرت نتائج الدراسة وجود تشوهات في كروموسومات الأشخاص الذين يتعرضون للملوثات مقارنة بالعينة الضابطة وبفرق ذات دلالة إحصائية ($p<0.05$). ومن أنواع التشوهات التي لوحظت في كروموسومات "عينة الدراسة: كسور في أحد أو كلا كروماتيدات الكروموسومات (chromatid breaks, isochromatid breaks)، عدم ارتباط في مكونات الكروماتيدات (chromatid gaps, isochromatid gaps)، تبادل لقطع بين كروماتيدات الكروموسوم (chromatid exchange).

خلصت هذه الدراسة وبشكل واضح إلى أن التعرض المباشر للملوثات البيئية هو مسبب رئيس لتشوهات كروموسومات الإنسان ومن الممكن أن يساهم في حدوث أمراض خطيرة كالسرطانات.وتوصي الدراسة بإلزام العاملين وأصحاب العمل في هذه المهن باستخدام وسائل الوقاية الضرورية لمنع التعرض لتلك الملوثات.

Introduction

Chromosomal aberrations in human peripheral blood lymphocytes are well-established biomarkers of exposure to occupational and other environmental genotoxic agents [1-4]. Cytogenetic analyses from persons occupationally exposed to such agents have shown increased frequencies of chromosomal damage [5-8].

Workers in many fields are occupationally exposed to various and complex mixtures of chemicals (synthetic and natural organic solvents, heavy metals, fuel emission particles), many of which were documented as mutagens [3,4, 9-12].Workers in Gaza Strip normally work without appropriate protective equipment, and the pollutants in many work places are not disposed of properly.

Chromosomal Aberrations in Males Occupationally Exposed to

Despite the fact that there are different occupations in Gaza Strip in which workers are exposed to harmful chemicals and pollutants, no previous studies have been conducted to evaluate the genotoxic effect of those compounds.
Here we report the results of the cytogenetic analysis of chromosomal aberrations in peripheral blood lymphocytes from a group of workers exposed to various chemicals as compared to a control group not directly exposed to such chemicals in their daily work.

Materials and Methods

Study subjects

The "study group" consisted of 32 males with a mean age of 35.5 years they are14 farmers, 3 plumbers, 5 taxi drivers, 6 paints factory workers, and 4 gas station workers.
The "control group" included 10 males' university employees with a mean age of 34.5 years, who were not directly exposed to chemicals or pollutants in the course of their professional work.
The objective of the study was first explained to all participants, after which they gave their informed consent. Blood samples (2 ml) were collected in heparinized tubes. Each individual also completed a personal history questionnaire on standard demographic questions such as age and place of birth as well as lifestyle and health status factors including employment record, time of exposure to pollutants, drug and tobacco use and eating habits.

Peripheral blood lymphocyte chromosomes preparation

Chromosomes were prepared from peripheral blood samples using standard methods [13]. Briefly, 0.5 ml heparinized blood was added to 10 ml complete RPMI-1640 in a sterile Falcon culture tube. For induction of lymphocyte proliferation, 2% (w/v) phytohemagglutinin-M was added to each culture tube. Tubes were incubated for 72h at 37ºC under 5% CO_2. Lymphocytes were arrested at metaphase by addition of colchicine (20 μg/ml) 30 minutes prior to harvesting of cells. Cells were collected by centrifugation, resuspended in pre-warmed hypotonic solution (0.075 M KCl), fixed in methanol: acetic acid (3:1), and spread on pre-cleaned microscope slides. The preparations were kept at 80 ºC for 72h and stained with 2.5% (w/v) Giemsa in methanol.

Evaluation of chromosome aberrations

The evaluation of chromosomal aberrations was performed following the IPCS (International Program Chemical Safety) guidelines for the monitoring of genotoxic effects of carcinogens in humans [13]. All slides was coded, and analyzed blind, with 100 well-spread metaphases screened for each individual. Achromatic lesions (gaps) were not considered as chromosomal aberrations [14]. Single chromatid and iso-chromatid deletions were included into one category as "chromatid(s) deletion". Chromosomal aberration frequency differences were analyzed using the Mann-Whitney Test [15].

Results

Data on the total number, type and frequency of chromosomal aberrations observed in the "study group" as compared to the "control group" is presented in table.1. Significantly higher incidence (2.41%, $p<0.05$) of chromosomal aberrations (chromatid(s) deletions, chromatid and iso-chromatid breaks, and acentric fragments) were detected in occupationally exposed workers as compared to the control subjects. The most frequent aberrations encountered in the "study group" were chromatid deletions.

Figure.1 shows representative microscope (1000x) photographs for each type of the aberrations documented in the study. Results of the number and frequency of chromosomal aberrations for each occupation in the "study group" are provided in table.2. As can be seen from the table, the highest frequency of aberrations were observed in plumbers who are occupationally exposed to various potential genotoxic substances including organic solvents and paints dust that are known to contain heavy metals. This category was followed by farmers who are exposed to various chemicals in the form of pesticides and fertilizers most of which are of proven toxicity to humans.

The most frequent types of aberrations differed between the categories e.g., in farmers more chromatid breaks were observed as compared to plumbers while more frequent chromatid(s) deletions were encountered in the taxi drivers. This may reflect the different modes of action of chemicals to which the workers are exposed to. This observation deserves further investigation.

Table.3 illustrates the frequency of chromosomal aberration in relation to exposure time. Interestingly, plumbers and farmers who showed significantly higher aberrations were occupationally exposed to pollutants for longer times (24 and 17.5 years, respectively) as compared to workers in other professions.

Discussion

Although chromosomal aberrations were observed in all categories of workers, only farmers and plumbers showed significant differences in the frequency of aberrations as compared to the control group. This may indicate that those two categories of workers are occupationally exposed to more genotoxic chemicals than others over a longer period of time. Verification of the nature of the genotoxic component(s) will be considered in a future study.

Analysis of questionnaire data showed that all workers do not use personal protective equipment, such as overalls, helmets and masks, and safety boots, and that most of the workers eat and drink right on the spot of the working area. These acts allow for the direct exposure to the chemicals handled in their work professions. Moreover, workers are not aware of the dangerous consequences of exposure to the chemicals they are using. The workplaces also lack the necessary safety measures for the disposal of the harmful chemicals, thereby, increasing the risk of exposure to those chemicals. Finding a significantly higher incidence of chromosomal aberrations in the lymphocytes of the "study group" as compared to the "control group", and an association between the frequency of those aberrations and time of exposure indicate that the observed increase in aberrations could be the primary consequence of occupational exposure to genotoxic compounds. This conclusion is congruent with various earlier studies in this field [1, 7, 10, 16].

Several authors consider cytogenetic biomonitoring (such as measurement of chromosomal aberrations) as a valuable index of exposure to genotoxic carcinogens and as a predictor of cancer risk [17-19]. This is plausible since chromosomal abnormalities are implicated in initiation and progression of many cancers. This study constitutes the first published record of occupational exposure to genotoxic agents in the Gaza Strip. Further studies are needed to analyze the nature of the chemical components that cause chromosomal damage, the mode of action of those chemicals on the genetic material, and link the various chromosomal aberrations with increased risk of cancer and other diseases such as immune system disorders.

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Table.1. Total number, type and frequency of chromosomal aberrations.

Group	Chromatid (s) deletions	Chromatid breaks	Iso-Chromatid breaks	Acentric fragments	Total aberrations
"Study group" (n=32)	32 (1.0%)	24 (0.75%)	7 (0.22%)	14 (0.44%)	77 (2.41%)
"Control group" (n=10)	0	0	0	0	0

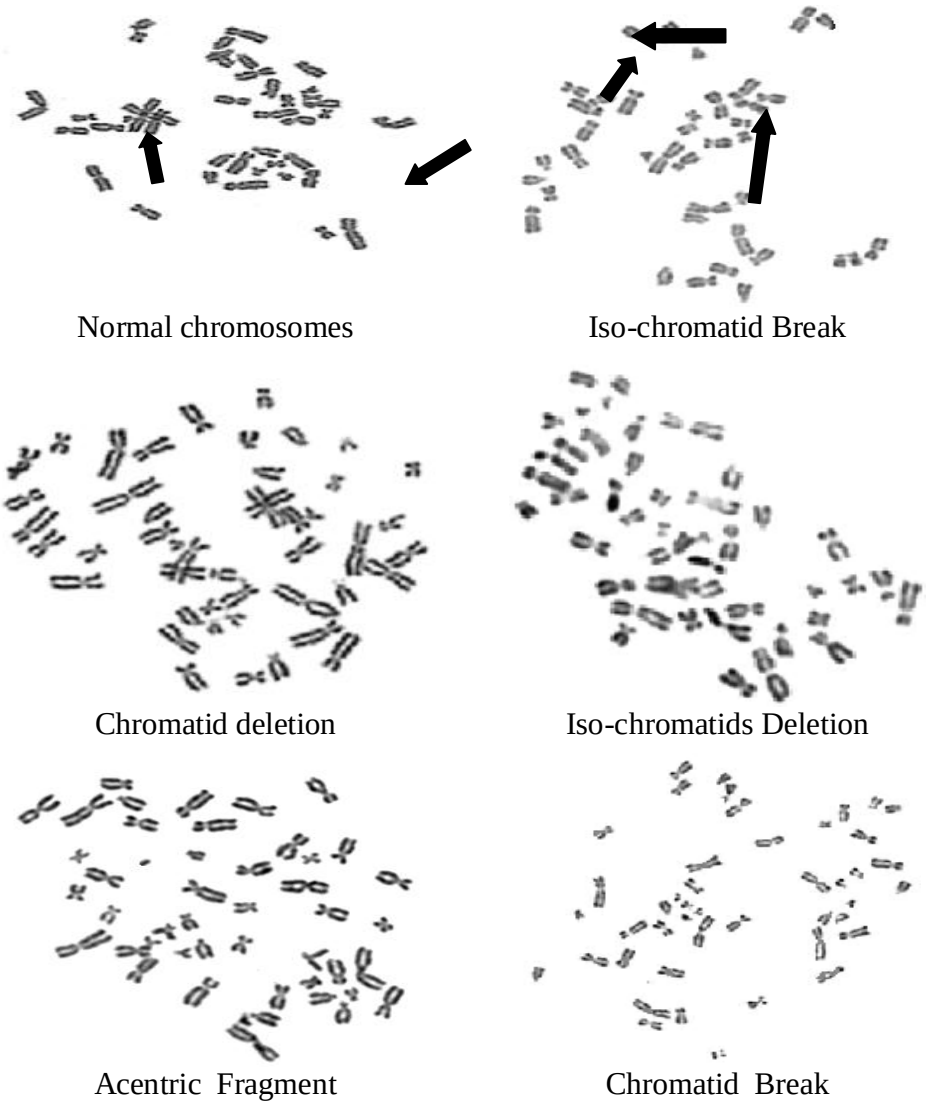

Figure 1. Representative microscope (1000x) photograph of the various chromosomal aberrations observed in the study. Arrow heads indicate the abnormality in each photo. Arrow heads do not appear in the photographs.

Table.2. Types and frequencies of chromosomal aberrations from categories of workers included in the study.

Occupation	Chromatid deletion	Chromatid breaks	Iso-Chromatid breaks	Acentric fragments	Total aberration per occupation	P value
Farmer (n=14)	7 (0.5%)	15 (1.07%)	3 (0.21%)	7 (0.5%)	32 (2.27%)	0.000*
Plumber (n=3)	5 (1.66%)	4 (1.33%)	2 (0.66%)	2 (0.66%)	13 (4.33%)	0.001*
Taxi driver (n=5)	11 (2.2%)	3 (0.6%)	2 (0.4%)	1 (0.2%)	17 (3.4%)	0.000*
Paints worker (n=6)	9 (1.5%)	0	0	0	9 (1.5%)	0.004*
Gas station worker (n=4)	0	2 (0.5%)	0	4 (1%)	6 (1.5%)	0.003*
Control group (n=10)	0	0	0	0	0	-

*: Statistically significant (Sig < 0.05).

Table.3. Frequency of chromosomal aberrations (all types) in relation to exposure time.

Occupation	Chromosomal aberrations	Mean Exposure time (years)
Farmer (n=14)	32 (2.27%)	17.5
Plumber (n=3)	13 (4.33%)	24
Taxi driver (n=5)	17 (3.4%)	14
Paints worker (n=6)	9 (1.5%)	5
Gas station worker (n=4)	6 (1.5%)	9