High Incidence of Chromosome Aberrations after Radiochemotherapy for Hodgkin's Disease: a Report of a Case and a Review of the Literature

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Abstract. To emphasize the importance of information obtained by conventional cytogenetic tests, in this study we will present a case report showing the development of HD in a young engineer of medical radiology, who had been professionally exposed to ionizing radiation during 4 years. Bio-dosimeter data and chromosomal aberration analysis made in a group of colleagues working in the same ionizing conditions excluded the possibility that she was overexposed to ionizing radiation, but retroactive analysis showed that at the time of employment she had a moderately increased level of chromosomal aberrations in peripheral blood lymphocytes (4.6%), with higher than normal incidence of chromatid breaks (22×10^{-3} per cell) and dicentric fragments (5×10^{-3} per cell). After the treatment of lymphoma by chemotherapy in combination with radiotherapy she also demonstrated a very high post-therapeutic level of chromosomal aberrations (21.5%), which consisted mostly of increased dicentric fragments (65×10^{-3} per cell). Although her illness is now clinically cured, the observed genotoxic changes point to a greater risk for delayed complications after HD, emphasizing the necessity for further continuous survey of this patient.

One of the scopes of preventive medicine is to find the ways to escape or prevent the appearance of a disease. Owing to this, one of the tests recommended by health care units and occupational medicine at the moment of employment of the person is chromosome aberration analysis of his/her peripheral blood lymphocytes. This test is the most sensitive method to estimate the genotoxic effects of various exogenous or endogenous agents able to damage DNA and induce numerical and structural changes of chromosomes, which later may provoke tumorigenesis and induce development of other diseases (Natarajan et al., 1996). Usually, the incidence of genome damage is not high in the whole population, although it is known that all people are constantly exposed to low background ionizing radiation, coming from cosmic radiation, rocks and soil or some radioactive forms of chemical elements (Little, 2000), as well as to non-ionizing radiation, linked mostly with the exposure to electric and magnetic fields (Campion, 1997; McCann et al., 1998). The genotoxic effects also depend on the sex, age and individual habits of examinees, such as cigarette smoking, alcoholism, use of drugs, etc. (Kasuba et al., 1995). Extremely dangerous effects, however, are caused by continuous exposure to ionizing radiation, which may induce permanent alterations in the DNA structure (Lloyd et al., 1980; Paz-y-Mino et al., 1995; Little, 2000). Owing to this, medical staff working in hospitals, where they are professionally exposed to X and gamma rays, neutrons, or electrons and alpha particles, is in obligation by the law to regularly perform conventional chromosome aberration analysis.

To emphasize the importance of information obtained by conventional cytogenetic tests, in this study we will present a case report showing the development of Hodgkin's disease (HD) in a young engineer of medical radiology, who had been working, during 4 years, in conditions with completely permissible exposure to ionizing radiation. However, at the time of employment, she had a moderately increased level of chromosomal aberrations in peripheral blood lymphocytes (4.6%), and after the treatment of lymphoma by chemotherapy in combination with radiotherapy she demonstrated a very high chromosome instability (21.5%). Although her illness is now clinically cured, the observed genotoxic changes point to a greater risk for delayed complications after HD, emphasizing the necessity for further continuous survey of this patient.

Material and Methods

Chromosome aberration analysis

A genotoxic analysis was performed by conventional metaphase analysis of peripheral blood lymphocytes, which were stained by Giemsa staining techniques

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Abbreviation: HD - Hodgkin's disease.

(Kasuba et al., 1995; Lalić et al., 2001). Briefly, short-term lymphocyte cultures were prepared using Gibco F10 medium, which was supplemented with 20% foetal calf serum, antibiotics and phytohaemagglutinin (Murex, Biotech ltd., Dartford, England). Two cultures of each sample were prepared. The cells were harvested at 48 h following stimulation. Colchicine (0.004%) (Sigma, Chemical Co., St. Louis, MO) was added 3 h before harvest. The cultures were centrifuged and subjected to a hypotonic shock (20 min, 0.075 M KCl) at 37°C. The lymphocytes were then fixed in acetic acid-methanol (1 : 3) and air-dried with 5% aqueous Giemsa solution for 10 min. In each person, 200 metaphases were analysed, seeking for structural aberrations such as chromatid and chromosome breaks, acentric and dicentric fragments, as well as for minutes and gaps.

Patient and disease history

The female patient, 25 years old engineer of medical radiology, was working during 4 years at the Radiology Department of Clinical Hospital Centre, Rijeka. One and a half year ago she noticed palpable lymph nodes on her neck. Biopsy and other tests confirmed HD CS IIA. Therapy was performed in Aviano, Italy, where she was receiving chemotherapy during 4 months in 8 cycles, which consisted of velbe, adriamycin, dacarbazin and bleomycin. The last cycle of chemotherapy was 1 year ago. Six months ago, she also received 20 cycles of irradiation with 40 Gy. Clinically, her illness is now completely eliminated, but the conventional genotoxic test revealed a high incidence of chromosomal aberrations. Regularly performed biodosimeter analysis also showed that her total absorption dose (7650 μSv), as well as annual absorption dose of radiation (3540 μSv), were not greater than permitted limits (20000 μSv and 3000-5000 μSv, respectively). Retroactive analysis showed, however, that at the moment of employment she had a moderately increased level of chromosomal aberrations in her lymphocytes.

Results

The data presented in Fig. 1 show multiple chromosomal aberrations found by conventional metaphase analysis in peripheral blood lymphocytes of the reported patient after combined radio-chemotherapy. Table 1 demonstrates the type of chromosomal aberrations found in 200 peripheral blood lymphocytes in metaphases at the moment of the patient's employment (4 years before the development of HD) and after chemotherapy and irradiation. As may be seen, at the moment when she started to work in ionizing conditions, the incidence of her chromosomal aberrations was 4.6%. Chromatid breaks represented the dominant type of aberrations (4.4 in 200 metaphases), but an increased number of dicentric fragments (0.8) was also found. One year after chemotherapy (consisting of a 4-month treatment with velbe, adriamycin, dacarbazin and bleomycin) and 6 months after 20 treatments of irradiation with 40 Gy, 21.5% of chromosomal aberrations were found. Dominantly found aberrations were minutes (26.0) and dicentric fragments (13.0). These data, collected of 200 metaphases, were also expressed per cell to compare the average values for chromosome aberrations found in this case with those found in two control groups of persons. Since she was working as an engineer of medical radiology in ionizing conditions during 4 years, one control consisted of 25 medical professionals who, during a prolonged period of time, worked in ionizing conditions in the same hospital (Lalić, 2001). The other control consisted of unexposed individuals discussed in previous reports (Lloyd et al., 1980; Gundy and Varga, 1983; Kasuba et al., 1995). The data showed (Fig. 2) that at the time of her employment the frequency of her chromatid breaks was 22.0×10^{-3}, which is several times greater than values found in both, exposed (3.8×10^{-3}) and unexposed (0.26×10^{-3}) persons. After therapy, however, a very high frequency of dicentric fragments was noticed (65.0×10^{-3}) in comparison with both controls (4.8×10^{-3} and 0.52×10^{-3}, respectively).

Discussion

Largely successful treatment of HD is one of the triumphs of modern cancer therapeutics. The death rate in the last 30 years has decreased by two thirds because of the introduction of effective diagnostic and therapeutic modalities (Harris, 1999; Diehl and Josting, 2000). However, despite the advances of recent years, many questions remain to be answered. One of them is connected with the possible serious late effects of aggressive therapy, since it was noticed that radiation and chemotherapy might result in excellent cure rates of HD, but also in especially high rates of second solid tumours or leukaemias (Cadman et al., 1977; Mendenhall, 1999; Clemons et al., 2000; Linassier et al., 2000) Although the aetiology of these radiation- or chemotherapy-induced cancers is multifactorial and incompletely understood, a vast amount of literature indicates that they might be the consequence of genotoxic effects of alkylating agents and DNA topoisomerase II poisons, procarbazine and irradiation, which are all leukaemogenic and immunosuppressive in animals and man (Stein at al., 2000; Pui and Relling, 2000). In several cases of drug-induced acute myeloid or promyelocytic leukaemia, the 11q23 mutation or t(15;17) were noticed more frequently (Gillis et al., 1995; Taylor et al., 2000; Zompi et al., 2000), but commonly, in HD most karyotypes are complexly aberrant, and although some chromosome regions seem to be preferentially involved, a chromosome aberration specific for HD has not yet been determined (Deerberg-Wittram et al., 1996). More frequently, deletions of 1p, 6q, and 7q were seen as well as deletions of 4q, with loss of 4q25 —> q27 (Atkin, 1998). However, evidence also shows that chromosomal