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ARTICLE

Is Breast Cancer Cluster Influenced by Environmental and Occupational Factors Among Hospital Nurses in Hungary?

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An unusual cluster of 8 breast cancer and 8 other malignant tumor cases (ovarian, uterus, lung, colon and brain tumors and malignant melanoma) developed in a period of 12 years among 98 nurses exposed to ethylene oxide (EtOx) for 5–15 years in a unit using gas sterilizer in a hospital of the archiepiscopal city of Eger, Hungary. EtOx concentration in air samples of the working area varied from 5 to 150 mg/m^3. The question was, if there was any causal relationship between the elevated incidence of breast cancer and the EtOx exposure, the other possibility was, that this cluster appeared accidentally. EtOx is a human carcinogen, however, no increased breast cancer incidence in EtOx-exposed subjects was reported in the literature. We followed up for two consecutive years the 27 non cancer patients, EtOx-exposed nurses and 11 unexposed hospital controls with the aid of a multiple genotoxicology monitor including chromosomal aberration, sister-chromatide exchange, HPRT point mutation and DNA repair studies. The results were compared with data from 30 local historical controls, 48 historical controls from Budapest, 14 hospital controls and 9 EtOx exposed nurses from Budapest. Significantly high chromosome aberration yields (especially chromosome type exchanges) were alike detected in EtOx-exposed and the two other control groups in Eger. These results could not be interpreted as a consequence of EtOx exposure only, since in the EtOx-exposed group from Budapest, beside an increased total aberration frequency, the obtained exchange type aberration yields were as low as the historical controls. A plausible explanation can be the natural low dose radioactivity (^{222}Rn) of the local tap-water due to a specific geological situation in Eger. The spontaneous breast cancer incidence in Hungary doubled in the last 10 years compared with the previous 20 years (1960–1980), especially in Eger. The appearence of the high breast cancer incidence in the hospital of Eger indicates the combined effect of EtOx and a more common local etiologic factor, such as the naturally radioactive tap-water. However, since the reported studies did not involve the investigation either of the genetic predisposition, or the effects of other possible environmental, occupational, and/or life style confounding factors, further studies (partly in progress) are necessary to clarify the importance of these factors. (Pathology Oncology Research Vol 5, No 2, 117–121, 1999)

Keywords: ethylene oxide, exchange aberrations, genotoxicology monitoring, ionizing radiation, risk assessment

Introduction

Breast cancer is a common disease among women in the civilized World. The cancer mortality rate in Hungary is the highest among 50 countries presenting data to the WHO statistics (i.e., more than 30,000 cancer death cases annually including 2,000 women with breast cancer, compared with the 10 million inhabitants).[1] Since 1950, breast cancer has become the number one cancer death cause of Hungarian females, and breast cancer incidence has increased from 16 to 32 per 100,000 cases between 1970 and 1990.[2,3] Among other factors, serious environmental deterioration and drastic changes in life-styles characterized Hungary during the last four decades increasing the environmental exposure to genotoxic noxa and changing the hormonal homeostasis. Epidemiological data demonstrate that environmental exposures have defined biological potential e.g., via reproductive hormones to initiate the carcinogenesis in the breast. However, only a few specific environmental exposures may be linked to breast cancer incidence, and no more than 50% of the cases can be

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directly explained by environmental risk factors.[4] Ionizing irradiation is a potent etiologic factor of breast cancer. Puberty, when the breast tissue develops rapidly, is the most sensitive age of cancer initiation, and an exposure to ionizing radiation in the age of 10-16 years causes more than twice as high risk for breast cancer in teenagers as in women older than 40 years.[5]

In this paper we report an unusual cluster of breast cancer incidence that appeared among nurses in the newborn unit of the county hospital in Eger.[6] The whole staff (i.e. both cancer patients and non patients) of the unit was exposed to ethylene oxide (EtOx, CAS Reg. No. 75–21–8) using a gas-sterilizer equipment for more than 12 years. The sterilizer was inappropriately controlled, repaired and run, so that high EtOx emissions occurred during sterilization twice daily. The question put by hospital officials was whether there was any relationship between the elevated incidence of breast cancer and the EtOx exposure, or had the cluster appeared accidentally? A causal relationship between EtOx and breast cancer would have broad implications in Hungary involving more than a thousand nurses using EtOx gas sterilizers in the country. These nurses would than need primary cancer prevention. The term "primary cancer prevention" refers to research investigating the means of a specific intervention with a measurable impact on cancer incidence in order to reduce the cancer risk, morbidity and mortality in populations exposed to environmental and/or occupational carcinogens.

Biological markers as e.g., chromosomal aberrations (CA) indicating the increased risk[7,8] are present in the early stage of the initiation of tumor development and are detectable in different non-target tissues, including peripheral blood lymphocytes (PBL). In the frame of primary cancer prevention, the use of cytogenetic end-points is the most feasible approach for occupational genotoxicological risk assessment.[9] Although ETO is a human carcinogen, no increased breast cancer incidence in ETO-exposed subjects has been reported in the literature.[10] Another possible environmental genotoxic factor was the temporary low natural ^{222}Rn activity in the local tap-water[11] of Eger. Radioactive radon exposure is a well known etiologic factor of breast cancer.[12] An additive genotoxic effect of chronic low dose radon and high dose ETO exposure could be a possible explanation for the occurrence of the cancer cluster in the particular hospital unit.

In order to investigate the putative additive effect of radon and EtOx exposures, and its significance in the cluster formation, we examined the healthy (non cancer patient) hospital unit staff by a routine multiple end-point genotoxicology monitor[13] including a cytogenetic investigation of PBLs. The results were compared with data from a local historical and a hospital control group, as well as with data from another EtOx-exposed group of nurses (hospital and historical control populations) in another town (Budapest) without excess natural tap-water radioactivity. The sterilizer had been removed, and we repeated the study in Eger among the available EtOx-exposed and hospital control donors a year later. Here we present the preliminary data underlying the necessity of further studies including investigation of the effects of the genetic predisposition, and of other environmental, occupational, and life-style confounding factors.

Materials and Methods

Sample selection

One hundred and four female donors – 27 high-dose ETO-exposed nurses in a county hospital in Eger, Hungary (mean age ± SE: 43.2 ± 1.2 years), 9 low-dose EtOxexposed nurses form a hospital in Budapest, Hungary (40.7 ± 3.3 years); 48 historical controls of Budapest without any known exposure (39.9 ± 1.1 years); 14 hospital controls of Budapest (27.5 ± 3.1 years); and 11 hospital controls of the investigated hospital in Eger (42.0 ± 3.8 years) – were involved in the first study.[10] Nurses in Eger with known and treated neoplastic disease were excluded. The same nurses from Eger were repeatedly investigated one year later: 25 EtOx-exposed nurses, 11 local hospital controls, and additionally 30 local historical controls (40.0 ± 1.5 years) without any known exposure. All individuals were interviewed about demographic data, smoking and drinking habits, exposure to ionizing radiation and/or to known, or suspected chemical mutagens, diseases, occupational history including duration of exposure to chemicals and the use of protective devices during work. Blood samples were collected from each donor by venipuncture with the donors' written permission for cytogenetic analysis and for routine clinical analysis.

Cytogenetic analysis (CA)

Whole blood samples were processed for CA: 0.8 ml samples of heparinized blood were cultured in duplicates in 10 ml RPMI-1640 medium (Gibco) supplemented with 20% fetal calf serum (Flow) and 0.5% Phytohemagglutinin-P (Difco), without antibiotics. The cultures were incubated at 37°C, in 7% CO_2, for 50 h, and 5 μg/ml 5-Bromo-2-deoxy-uridine (BrdU, Sigma) was added at 0 h of incubation. Culture harvest, slide preparation and staining were performed following the standard method of Moorhead et al.[14] including a Fluorescent-Plus-Giemsa staining[15] in order to study only the first metaphases. All microscope analyses were performed on coded slides by the same two observers. CAs were characterized in at least one hundred metaphases with 46 ± 1 chromosomes per subject according to Carrano and Natarajan.[16] Mitoses containing only achromatic lesions (gaps) and/or aneu-

ploidy, were not considered to be aberrant. The significance of CA yield differences between the control and exposed groups was analyzed by the Wilcoxon test. $P<0.05$ was the limit of significance.

Results and Discussion

In the investigated hospital unit 14 cancer patients, i.e., 8 breast cancer (up to date 4 death tolls, minimum and maximum age: 45 and 51 years, respectively) and 8 other malignant tumor cases, i.e., ovarian, uterus, lung, colon and brain tumors, and malignant melanoma (up to date 3 death tolls, minimum and maximum age: 49 and 54 years, respectively) occurred among the staff persons in a period of 12 years.[6] EtOx concentration in air samples of the working area varied from 5 to 150 mg/m^3 daily.[17]

CA results obtained in each investigated group are summarized on *Table 1*. In another study, Lerda and Rizzi found no significant changes in CA yields in PBLs of EtOx-exposed donors 3 months after removal from work indicating a long term effect of EtOx exposure.[18] In the first study we observed significantly increased CA frequencies including high exchange aberration yields in EtOx exposed group without neoplastic diseases in Eger, when compared with another EtOx exposed control group in Budapest.[10] We repeated the study a year later, after the sterilizer had been removed already for one and a half years expecting a significant decrease in CA yields.[19] Although CA frequencies were lower during the repeated study compared with the previous results, the CA yields, especially the exchange aberration frequencies were still significantly higher than in EtOx-exposed nurses in Budapest. Similarly, in the literature only slight increase of exchange aberration frequencies have been reported in EtOx-exposed subjects.[10] We also observed an increased CA frequency including exchange aberrations in the local hospital controls in Eger, without any recorded occupational exposure. Consequently, the obtained CA yields in Eger cannot be explained by EtOx exposure alone. Additional possible carcinogenic factors occurring in the investigated unit, like X-ray, UV-light, NaOCl, or the formation of epoxy compounds from EtOx and NaOCl in the presence of UV light in the working environment were also studied, but excluded as factors responsible for the obtained cytogenetic changes because of the obtained negative results. The high CA level among hospital controls at the same time suggested a possible genotoxic factor other than EtOx,[10] and the data indicate that occupational EtOx

Table 1. **Cytogenetic results obtained in historical and hospital control and ETO-exposed groups (females in reproductive age)**

Groups	*n*	*Total aberrations*	*Chromatid type aberrations*	*Chromosome type aberrations*	*Total exchanges*	*References*
		Mean % ± SE[1]				
Historical control (Budapest, cumulated)	74	0.34 ± 0.11	0.18 ± 0.08	0.16 ± 0.07	0	10
Local control (Eger, 1996)	30	3.66 ± 0.46[2]	1.70 ± 0.31[2]	1.97 ± 0.32[2]	0.67 ± 0.20[2]	6
Hospital control (Eger, 1993)	10	5.81 ± 0.92[2]	3.10 ± 0.65[2]	2.71 ± 0.58[2]	1.88 ± 0.31[2]	10
Hospital control (Eger, 1994)	11	4.42 ± 0.84	1.42 ± 0.31	3.00 ± 0.67	1.08 ± 0.23	19
EtOx-exposed nurses (Budapest, 1993)	9	4.66 ± 0.89 [2]	1.77 ± 0.33[2]	2.88 ± 0.32[2]	1.32 ± 0.25[2]	10
EtOx-exposed nurses[4] (Eger, 1993)	27	8.59 ± 0.92[2,3]	4.37 ± 0.65[2,3]	4.22 ± 0.63[2,3]	2.57 ± 0.61[2,3]	10
EtOx-exposed nurses[4]	25	5.16 ± 0.52[2]	1.80 ± 0.33[2]	3.36 ± 0.44[2]	1.24 ± 0.22[2]	19

[1] Standard error (SE)
[2] Significant to the historical control ($p<0.01$)
[3] Significant to EtOx-exposed, Budapest ($p<0.05$)
[4] Exposure to EtOx was terminated in 1992

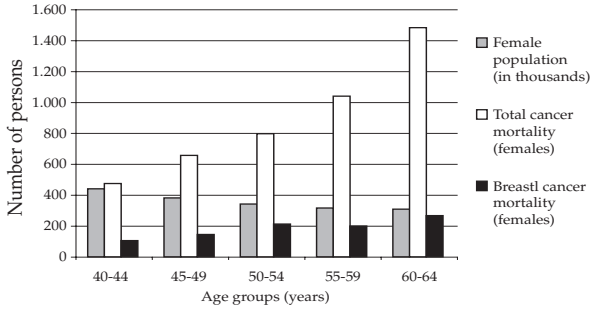

Figure 1. *Breast cancer mortality in age groups for Hungary in 1996.*

exposure is only one of the several possible factors which promoted this (breast) cancer cluster outbreak in Eger.

As it is well known from the World cancer statistics,[1] both the breast cancer mortality and morbidity have been increased in the civilized World, especially in the age group of 45-54 years. The same tendency in Hungary is proved by the statistical data.[20] The breast cancer mortality data of age groups for Hungary in 1996 are shown in *Figure 1*. In Eger, the breast cancer incidence is higher than the average incidence in Hungary.[21] Analyzing the reasons we found no increase in the average air pollution, or in the pesticide contents in the local vegetables and different food supplies above the acceptable level.[22,23] Environmental genotoxic noxa as etiologic factors were still obvious, since the occupational EtOx exposure was shown by the cytogenetic monitor, and there were increased CA yields among the local controls as well. However, no evidence has also been reported on increased breast cancer incidence among ETO-exposed workers,[24] and nurses[25] in the literature. Consequently, the clustering occurrence of breast cancers among nurses in Eger could rather be produced by the additive effect of EtOx and another undefined genotoxic factor, than by EtOx alone. On the other hand, there was no genetic relationship between the cancer patients, and the anamnestic data only showed familiar appearence in two cases. However, interaction between the genetic predisposition and the environmental noxa cannot be excluded and needs further studies.

Ionizing radiation is also an established etiologic factor of breast cancers.[12] A possible environmental genotoxic factor could be the excess ^{222}Rn concentration in the local tap-water present in Eger for decades.[11,26,27] The city of Eger lies on a geological fracture zone separating a Carboniferous-Permian limestone massive and a Miocene volcano. Certain local sunk wells producing slightly radioactive water from the eruptive layers, serving mainly the thermal baths, are temporally connected into the drinking water supply system of the city. Radioactive water is mixed with inactive one from other wells in a temporarily changing rate. In the years 1963–1976, the measured ^{226}Ra, and ^{222}Rn activities of the local tap water samples in Eger were 11–210 mBq/L, and 3.7–114.7 Bq/L, respectively,[24] and in 1993 the measured ^{222}Rn activities were in the range of 2.7-51.0 Bq/L.[25] In the investigated EtOx-exposed group we found decreased white blood cell (WBC) counts, and analysis of variance indicated a significant ($p = 0.0128$) association of increased CA yields with a decreased WBC level.[10] Bojtor et al.[24] also reported decreased total WBC counts among 406 inhabitants drinking radioactive tap water in Eger. This means that the natural, slightly radioactive tap water in Eger must be considered a chronic genotoxic factor. The possible mechanisms of interaction between EtOx and chronic, low level, high linear energy transfer ionizing radiation leading to excess exchange aberration formation are discussed elsewhere.[19]

In conclusion, we suggest that occupational EtOx exposure could have served as an additive (confounding) genotoxic factor stimulating the clastogenicity of natural ^{222}Rn radioactivity that resulted in the outbreak of the (breast) cancer cluster in the hospital of Eger. However, since the reported studies did not involve the investigation either of the genetic predisposition, or the effects of other possible environmental, occupational, and/or life style confounding factors, further studies (partly in progress) are necessary to clarify the importance of these factors.

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