Human Germinal Mutagenic Effects in Relation to Intentional and Accidental Exposure to Toxic Agents

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This paper presents examples of epidemiological evaluation of exposure- and cluster-type mutations in human populations. The self-poisoning model did not show that offspring born from mothers after a semilethal self-poisoning had higher rates of prenatal selection (fetal death) or abnormalities due to germinal mutations; however, an intrauterine growth retardation was found. The surveillance function of the Hungarian Congenital Abnormality Registry has detected many cluster-type situations, one example of which is an extreme increase of Down's syndrome in a small Hungarian village in 1989 to 1990. Environmental investigations have pointed to the excessive use of trichlorfon at local fish farms as the cause. — Environ Health Perspect 104(Suppl 3):615–617 (1996)

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Introduction

In studies of human populations exposed to germinal mutagenic and reproductive hazards, there are two approaches: exposure-type situations and cluster-type situations (1). Exposure-type situations include atomic bomb victims in Japan, cancer patients with radiotherapy and/or chemotherapy, and persons who have attempted suicide with large doses of chemicals (the self-poisoning model). The self-poisoning model has some demographic and medical characteristics in Hungary: self-poisoning has risen dramatically in the last several years; it occurs predominantly in the young (the peak is at the age of 17–19 years) and more often in females (68%) than in males (32%); the proportion of survivors of self-poisoning has increased due to more effective medical treatment; and because patients are hospitalized, data are much easier to access. Thus, due to the availability of data, we have systematically studied the germinal mutagenic effects in individuals who attempted suicide using chemicals and drugs (2,3).

Cluster-type situations basically include registries of people with a particular health effect often related to a common exposure. One important registry in Hungary is the Hungarian Congenital Abnormality Registry. The Hungarian Congenital Abnormality Registry was established in 1962 as the first national-based registry in the world. This registry comprises all reported cases with congenital abnormalities diagnosed from terminated pregnancies due to prenatally diagnosed fetal defects in the second and third trimesters through 1 year of age. The surveillance function is one of the most important tasks of the registry, i.e., it is necessary to detect time and spatial clusters as soon as possible.

Methods and Materials

Exposure-type Situations

The study sample consisted of 1,395 persons under 30 years of age who attempted suicide using extremely large doses of chemicals, sufficient to cause at least 1 day of unconsciousness; these individuals had been medically treated in the toxicological department of Korányi Hospital, Budapest between 1960 and 1967. The control sample included 881 individuals who had undergone surgery for varicosity, appendicitis, and hernia in the surgical department of the same hospital over the same time period. They were matched for sex, age, and district of residence with the index cases. All male index cases could be matched, but each pair of index female cases had one matched control female. Reproductive data were obtained blindly in personal interviews of all index cases and matched controls during home visits; interviewers did not know whether the study person belonged to the self-poisoning group or the control group. Informed consent was obtained from participants in the study. The $X^2$, Fisher exact, and $U$ tests were used for statistical analysis.

Cluster-type Situations

The recorded rate of 50 congenital abnormality entities is evaluated each year in the 20 regional units of Hungary (19 counties and Budapest) and compared to the expected baseline rates, which are known due to ad hoc epidemiological studies. After the detection of a spatial increase of a given congenital abnormality in each of the above 20 regional units, all settlements of this unit are evaluated separately to localize its hot spot. If an area with an increase in a congenital abnormality is detected, a field study is organized. The first task is to check the diagnoses of the cases because the majority of spatial clusters are caused by technical biases (e.g., misdiagnoses). The second task is to organize a case–control study. In the 1980s, 54 spatial clusters were detected in Hungary. Some of them were related to Down's syndrome; one example of evaluation of such a spatial clustering is presented (4).

Results and Discussion

Exposure-type Outcome

Using the occurrence of pregnancies as an indicator of fertility in 1,036 index females after self-poisoning (2), two different pregnancy outcomes (live birth and fetal death) and congenital abnormalities in live births did not show any significant difference compared with the data of matched controls (Table 1). The occurrence of pregnancy was lower ($X^2_{1} = 5.41; p = 0.02$) while the rate of fetal death was higher.
(χ² = 15.3; p < 0.001) in the wives of self-poisoned males than in wives of controls (Table 2); however, the latter figure was probably underdetected in the control group. The most sensitive phenotypic end points of germinal mutations (i.e., the indicator abnormalities) in the F₁ generation (5) did not show a significant increase (Table 3); however, a much larger sample (about 13,500 live births) is needed to detect a doubling of germinal mutations. Birth weight and gestation age were also evaluated in live-born infants of self-poisoning individuals. Babies born to self-poisoning females months or years after an attempted suicide were found to have lower birth weights than babies born before the suicide attempt (Table 4). The difference in the birth weight of subsequent pregnancies was also highly significant between self-poisoning and control women. A similar, but not significant, trend was observed in the live births of female partners of self-poisoning males. Gestation age was not different between the study and control groups; thus, the lower birth weight must have been caused by intrauterine growth retardation. A similar observation was found in experimental studies after radiation (6) or chemical treatment (7).

It is not clear whether large semilethal doses of chemicals taken by self-poisoning persons can produce germinal mutations with epidemiologically detectable consequences in the F₁ generation. There is no evidence to indicate that semilethal self-poisoning increases the prenatal selection (fetal death) and indicator abnormalities of germinal mutations; however, intrauterine growth retardation was found. The message of our study is important from the medical point of view, but we need more sensitive methods, e.g., molecular epidemiology techniques, to provide definitive results.

**Cluster-type Outcome**

Of 15 infants born in 1989 and 1990 in a small Hungarian village (Rinyaszentkirály), 4 had Down's syndrome. Thus, the recorded rate (266.67/1,000) exceeded the expected rate (1.17/1,000) 228 times. Other unusual findings were noted: 7 other babies had congenital abnormalities, and of 15 live births, 6 (40%) were twins (although the expected rate was 2%). All four cases with Down's syndrome had trisomy 21, but their parents were healthy; however, the origin of an extra chromosome 21 was maternal meiosis II. This is in contrast to the typical nondisjunction that occurs in maternal meiosis I. Two complementary studies were therefore organized to investigate the phenomenon.

The clinical examination of all 61 children born in the village between 1980 and 1988 did not detect any occurrence of Down's syndrome. As a further check, we looked at the records for children born in the neighboring seven settlements less than 20 km from Rinyaszentkirály. Only one case of Down's syndrome was found, and the observed rate corresponded well with the expected baseline rate. Thus, we concluded that the spatial clustering of Down's syndrome was restricted to Rinyaszentkirály and to just the 2 years, 1989 and 1990.

Subsequently, we organized a case-control study. The involvement of classical hazardous environmental factors such as alcohol consumption, radiation, drug ingestion, microbial infections, maternal disorders, etc., were not found. The confounders, e.g., demographic factors such as maternal age, birth order, and previous fetal loss, also did not show any significant differences among groups studied. However, all mothers of Down's syndrome cases reported frequent fish consumption.

**Table 1.** The occurrence of pregnancies (fertility), pregnancy outcomes, and congenital abnormalities in live births in self-poisoning females (after self-poisoning) and their matched control groups.

| Variables          | Self-poisoning females (n = 1,036) | Matched controls (n = 518) |
|--------------------|----------------------------------|---------------------------|
| Number             | %                                | Number                    | %                       |
| Pregnancy          | 842                              | 81.3                      | 438                      | 84.6                    |
| Pregnancy outcomes |                                  |                           |                          |                         |
| Fetal death        | 204                              | 16.4                      | 88                       | 14.6                    |
| Live births        | 1,042                            | 83.6                      | 506                      | 85.2                    |
| Congenital anomalies | 31                               | 3.0                       | 11                       | 2.2                     |

**Table 2.** The occurrence of pregnancies, pregnancy outcomes, and congenital abnormalities in live births after self-poisoning in the wives of self-poisoning males and their matched controls.

| Variables          | Self-poisoning males (n = 363) | Matched controls (n = 363) |
|--------------------|--------------------------------|---------------------------|
| Number             | %                                | Number                    | %                       |
| Pregnancy          | 249                              | 68.6                      | 277                      | 76.3                    |
| Pregnancy outcomes |                                  |                           |                          |                         |
| Fetal death        | 48                               | 12.1                      | 24                       | 4.9                     |
| Live births        | 350                              | 87.9                      | 468                      | 95.1                    |
| Congenital anomalies | 7                               | 2.0                       | 6                        | 1.3                     |

**Table 3.** The occurrence of indicator abnormalities of germinal mutations after self-poisoning in the live births of self-poisoning individuals and their matched controls.

| Indicator abnormalities | Live births of self-poisoning Females (n = 1,042) | Live births of matched control Females (n = 506) |
|-------------------------|-----------------------------------------------|-----------------------------------------------|
|                         | Number | %       | Number | %       | Number | %       |
| Sentinel anomalies      | 1      | 0.1     | 0      | 0       | 0      | 0       |
| Down's syndrome         | 2      | 0.2     | 0      | 0       | 0      | 0       |
| Unidentified multiple   | 2      | 0.2     | 0      | 0       | 1      | 0.2     |
| congenital abnormalities|                                  |                                               |                         |

**Table 4.** Mean birth weight (g) of live-born infants from previous and subsequent pregnancies of self-poisoning females and males and their matched controls.

| Pregnancy       | Females | Males |
|-----------------|---------|-------|
|                 | Self-poisoned | Control | Self-poisoned | Control |
| Previous        | 3,131   | 3,151  | 3,083   | 3,151   |
| Subsequent      | 2,931   | 3,245  | 3,013   | 3,468   |
| Difference      | g       | <0.05  | <0.05   | >0.05   |
|                 | U       | <0.05  | <0.05   | <0.05   |
|                 | p       | >0.05  | >0.05   | >0.05   |
Follow-up investigations showed that the territory of Rinyaszentkirály includes several fish ponds, and a new director of fish farms introduced chemical fast bathing of fish in 1988. These fish were taken out of the ponds and treated with 500 mg/l Flibol (40% trichlorfon) for 5 to 10 min to eradicate parasites and then were returned to the pond. Trichlorfon is an organophosphorus insecticide, which is also known as chlorofos, metrifonate, and trichlorophane; brand names include Dipterex and Flibol. After the treatment, fish seemed to be lifeless on the surface of the water for hours. Dead fish were noted both by inhabitants and those supervising the cleaning operation. The inhabitants of Rinyaszentkirály were not told about the new technology, but fishing was prohibited during these periods (March and May in 1988 and 1989); however, people often took the comatose fish by hand for consumption in this impoverished village. The estimated initial trichlorfon content of the fish was about 100 mg/kg (acceptable daily intake is 0.01 mg/kg). All mothers of Down’s syndrome babies consumed poisoned fish during the critical period, i.e., on the day of conception. This chemical treatment of farmed fish was banned in 1990. All 30 children born after the ban between, 1991 and 1994, are healthy, thus the cluster ceased. In addition, Doherty et al. (8) confirmed the aneugenic effect of trichlorfon in experimental studies. Thus, the possibility that high-dose trichlorfon is a germinal mutagenic agent deserves further attention.

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