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Down's Syndrome and Malignancy

By

D. Schuler, Matilda Dobos, G. Fekete, T. Machay, Agnes Nemeskéri

Second Department of Paediatrics, and Second Department of Anatomy, Semmelweis Medical University, Budapest

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Although the principal causes of death in Down's syndrome are still infections and congenital heart defects, with the use of antibiotics and the possibility of surgical repair leukaemia has become increasingly significant in this respect. In the present study, the higher maternal age did not explain the frequency of leukaemia in children with Down's syndrome. Leukocytic NAD activity was also normal in the examined material. On the other hand, the chromosomes of Down patients displayed "in vitro" an increased mutability on treatment with chemical mutagens. It is suggested that an increased susceptibility to chromosomal mutations and non-disjunction contribute to the higher incidence of leukaemia in Down's syndrome.

Infectious diseases and congenital anomalies are the principal factors responsible for the high mortality of children with Down's syndrome [16]. The widespread employment of antibiotics and the possibility of repairing congenital malformations have considerably increased the significance of malignancies, of leukaemia in particular, as lethal factors in the syndrome [6].

The present study was designed to investigate the factors causing the death of children in Down's syndrome with special regard to the incidence of associated malignancies.

Material and Method

(1) Statistical data

(a) We analysed the post-mortem reports of the two institutes of pathology of Budapest University as well as those of the children's hospitals of Budapest from January 1, 1962 to December 31, 1970. We found only 40 cases of Down's syndrome which shows that many of such patients probably did not die in children's hospitals.

(b) With a view to elucidating the possible correlation between maternal age (which is significantly higher than the average in the case of children with Down's syndrome) and the occurrence of leukaemia, we recorded the age of the mothers and the birth order of their offspring in respect of 66 of those 108 children who had been treated with acute leukaemia at the 2nd Department of Paediatrics in the period 1949 to 1970. The data so obtained were then compared with those for the country's healthy population, recorded by the Central Statistical Office.

(c) We recorded the proportion of Down's syndrome in our material of leukaemic children as this disease is known to have a high incidence in cases of Down's syndrome [21, 24, 37].

(2) Chromosome studies

Lymphocyte cultures from the peripheral blood of four Down patients were examined after 72 hrs for chromosomal anomalies. Employing the method of MOORHEAD et al. [27] with some modification [29], we found one instance of D/G translocation and three of G-trisomy. In these four patients repeated chromosome examinations were performed by applying in vitro the alkylating agent tetramethane-sulphonyl-d-mannitol (Zitostop®). Earlier investigations [31] had namely shown that, applied to 72-hour cultures of human lymphoid cells at a concentration inhibiting about 50% of the mitoses, this agent gives rise to chromosome breakage and translocation. The compound dissolved in water was added at final concentrations of 0.01 μg/ml, 0.1 μg/ml and 1.0 μg/ml at the beginning of cultivation in some cases and after 48 hours in others. The cultures had a volume of 10 ml. (7.4 ml TC-199 [Difco] + 2 ml human plasma + 0.5 ml cell suspension + 0.08 ml PHA + 1000 U penicillin + 1.0 mg streptomycin.)

The ensuing chromosomal anomalies were studied in 1225 cells of 48 cultures. Only marked dislocation of the broken chromosomal end was classified as breakage so that achromatic gaps were disregarded.

(3) Enzyme studies

An accumulation of nicotinic acid amine dinucleotide (NAD) characterizes certain forms of acute childhood leukoses [5]. Since in the leukocytes several enzymatic anomalies were found in the cases of Down's syndrome [5, 12, 18], we examined the amount of NAD in the white blood cells of 10 children afflicted with the syndrome (NAD + ethyl alcohol—alcohol dehydrogenase—NAD—H_2 + acetaldehyde). White cells of healthy and leukaemic children served as controls.

RESULTS

(1) Distribution of the material according to the cause of death is shown in Table I. It can be seen that infections (pneumonia in particular) and congenital heart defects were the most frequent causes. They often appeared in combination: many fatal cases of pneumonia displayed some congenital heart defect, while pneumonia was invariably present in children who had succumbed to a heart defect. Our classification was

TABLE I

Causes of death

Infection	without congenital malformation	9
Congenital malformation	with infection	24
	without infection	4
Birth injury		1
Leukaemia		2
	Total	40

TABLE II

Incidence of Down's syndrome in leukaemic patients

Number of leukaemic children	543
Children with Down's syndrome and leukaemia	5
Incidence of Down's syndrome among leukaemics	0.92%
Incidence of Down's syndrome in the newborn population	0.07%

TABLE III

Chromosomal aberrations in the blood cultures of Down's syndrome patients after tetramethane-sulphonyl-d-mannitol treatment

Concentration of mutagen, μg/ml	Time of incubation, hrs	In Down's syndrome				Healthy controls	
		Total number of cells	Cells with chromosome breakage	Total number of breakages	Percentage of aberrations	Total number of cells	Percentage of aberrations
0.01	72	420	44	46	11.0	500	4
	24						
0.1	72	420	61	66	15.9	553	6
	24						
1.0	72	175	42	55	31.1	401	26
	24	210	46	51	24.2	253	10.5

graded according to the gravity of the latter condition. In two cases death was due to leukaemia; considering this low number, its occurrence in our material may have been a chance phenomenon. It seemed better to approach the problem from the opposite angle by ascertaining the incidence of Down's syndrome among leukaemic children. We succeeded in collecting data for 543 leukaemic children among whom 5 were suffering from Down's syndrome.* This means an incidence of 9.2‰ against the national average of 0.7‰ [17] (Table II).

(2) Chromosome breakage in Down's syndrome

No increase in the number of spontaneous chromosome breakage was observed; we examined therefore the number of mutations induced by Zitostop® in vitro. Results are listed in Table III.

It is clear that the alkylating agent increased the number of breaks in both the Down and the healthy children. The difference in the number of breaks between the Down cases and the control group was highly

* We are indebted to the First Department of Paediatrics, Semmelweis Medical University, the Pál Heim Hospital of Sick Children, Budapest; the Departments of Paediatrics of the Pécs and Szeged Medical Universities, for data placed at our disposal.

significant after the addition of Zitostop® at concentrations of 0.01 and 0.1 μg/ml, whereas the difference became significant after 24-hr incubation only if the concentration of the alkylating agent was 1.0 μg/ml ($p < 0.01$) (Fig. 1).

During the 12 years 1945 to 1956, the number of Hungarian children with Down's syndrome who reached the age of 6 to 7 years totalled 691 [17]. The mortality of school-age children with Down's disease was fairly constant over these years so

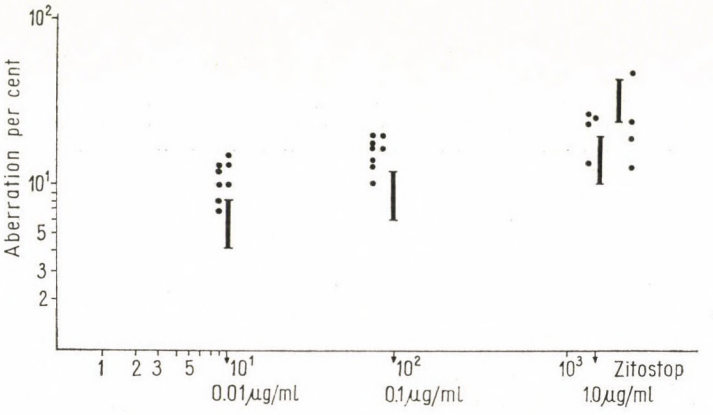

FIG. 1. Chromosomal aberrations after Zitostop treatment of leukocytic culture of children suffering from Down's syndrome — in comparison to healthy controls. Means and 95% confidence limits of the controls are represented by vertical lines

(3) Enzyme studies in Down's syndrome

The NAD content in the leukocytes of 20 children with Down's syndrome was invariably found normal. The values obtained as also those characteristic of leukaemia and the normal values are presented in Table IV.

DISCUSSION

The two points studied were,

(1) the cause of early death in cases of Down's syndrome;

(2) the cause of the association of leukaemia with Down's syndrome.

that it was possible to calculate the mortality of 6 to 16-year-old Down children with approximate accuracy. A total of 19 school children with Down's syndrome died during the 12 years; this meant a 2.7% mortality against the mean 0.43— for the same age group. The situation is even more grave if one considers that the mortality of Down's syndrome is highest among infants and young children. According to GITTELSON [10] only 50% of the children with Down's syndrome survive to school age.

Infections or their combination with congenital heart defects were in our material the principal cause of

TABLE IV

NAD activity in leukocytes in leukaemia, Down's-syndrome, and in healthy children

Disease	Number of cases	NAD $\mu g/10^9$ white cells
Acute myeloid leukaemia	9	92.6 ± 12.3
Acute lymphoid leukaemia	17	47.5 ± 11.3
Down's syndrome	20	48.5 ± 3.2
Normal leukocytes	6	48 ± 2.8

death during the period 1962—1970. For the frequency of infections, the diminished resistance of the organism in Down's syndrome may be responsible. The reduction of resistance has been attributed by certain workers to decreased antibody production [34]. Whether the degree of cellular immune response is lower or normal in Down's disease is still a question; in any case the immune globulin level is higher than normal in such patients [1a]. The use of antibiotics has reduced the lethality of infections and most heart defects are surgically repairable so that leukaemia is becoming increasingly significant as the cause of death.

The frequent association of Down's syndrome and leukaemia was pointed out by KRIWIT and GOOD [21a] who, in addition to their own case, knew of further seven such cases in the USA. FANCONI [7] found 7 cases of Down's syndrome among 185 leukaemic children (3.8%), and the incidence observed by STEWART et al. [36] was 2.6%. These figures indicate an even higher frequency than that observed in our material (Table II).

It was from three angles that we approached the question as to why the two conditions were so often associated.

(1) Does the fact that the mothers of children with Down's syndrome are

TABLE V

Occurrence of leukaemia and Down's syndrome in the same family

	Number of leukaemia cases	Number of Down's syndrome cases
Ebbin et al. (1960)	1	3
Buckton et al. (1961)	1	3
Thompson et al. (1963)	1	2
Conen et al. (1966)	1	2
Miller et al. (1963)	1	2
Holland et al. (1962)	2	2
Heath and Moloney (1965)	5	1

older than the average promote the development of leukaemia?

(2) Do the leukocytes of Down patients display metabolic or enzymatic irregularities characteristic of leukoses?

(3) Do the chromosomes show a sensitivity to chemical mutagens that would indicate an increased susceptibility to mutations?

ad (1). According to STARK and MANTEL [35] as well as to STEWART et al. [36], advanced maternal age facilitates the development of leukaemia in children with Down's syndrome. As regards our material it was in connection with 66 leukaemic children that we were able to establish their birth order and the age of the mother. The distribution of live births showed practically constant values in Hungary during recent years (1963—1967) and the tendency of distribution was much the same in our leukaemic material. Admitting that our 66 cases do not justify conclusions we suggest that the age of the mother and the birth order of their child is not in connection with the increased frequency of leukaemia in cases of Down's syndrome.

ad (2). The so-called leukaemoid reaction (which often differs from true leukaemia only by its reversibility) is known to occur frequently in newborn babies with Down's syndrome [38]. It is likewise known that the activity of certain enzymes is above normal in the leukocytes of Down patients, a phenomenon that may play some role in the development of leukaemia. NAD activity in the white cells of leukaemic children is abnormal [5]. MOLONEY and LANGE [26] who examined survivors of the atomic bomb explosion in Japan, observed biochemical changes in the leukocytes long before the leukaemia had manifested itself. It was for this reason, that we examined the activity of NAD in the leukocytes of our Down patients. We found it to be normal.

ad (3). Malignancies including leukaemia are usually accompanied by chromosomal aberrations. It is further known that diseases with increased number of spontaneous chromosome breakages (Fanconi's anaemia, ataxia teleangiectasia, Bloom's syndrome) are accompanied by malignant tumours and leukaemia at a higher than average frequency [9, 11, 15, 28, 30]. Increased spontaneous chromosomal fragility in cases of Down's syndrome was observed by KAHN and ABE [19]; they examined the chromosomes cultured from 75 fathers and 85 mothers of Down children and found the number of chromatid breakage three times higher (1.25%) than the normal value.

Chromosome breakage may be caused by diverse exogenous factors (X-rays, chemicals, viruses, physical influences). Chromosomal sensitivity to mutagens is increased in certain diseases and congenital anomalies, a phenomenon that presumably contributes to the frequency of malignancies [30, 33]. This assumption is supported by the observation that the lymphocyte culture of Down patients shows increased susceptibility to trans-

formation by the oncogenic virus SV 40 [40].

After such antecedents it was reasonable to think of the possibility that Down patients may be particularly sensitive to mutagens which would facilitate the development of leukaemia.

The children of our material showed no increased spontaneous chromosomal fragility, but Zitostop® considerably increased chromosomal mutability in their leukocytes. It is, therefore, probable that an increased susceptibility of the chromosomes to mutation or a reduced capacity for the repair of chromosomal aberrations may contribute to the frequency of leukaemia in cases of Down's syndrome.

A tendency toward irregular cell division, too, may be a contributing factor. This tendency is sometimes a familial trait as it appears from the reports on the association of Down's syndrome and leukaemia in the same family [2, 3, 4, 14, 16, 25, 39] (Table IV). However, other workers failed to support the existence of such a familial disposition after examining the siblings of leukaemic patients [1b]. The 21 trisomy itself predisposes to non-disjunction since, as pointed out by LEJEUNE [21b], all chromosomal aberrations pave the way for further aneuploidy which may lead to multiple aberrations, mosaicism and even leukaemia. We encountered in our material, in addition to 58 cases of trisomy, 5 cases of 46/47 mosaicism and one case of X + 21 double trisomy.

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Dr. D. Schuler
Tűzoltó u. 9
Budapest 9, Hungary