

Chromosomal aberrations in infective hepatitis

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SYNOPSIS Structural chromosomal aberrations, in the form of breaks, were found in a significantly higher proportion of bone marrow cells in patients with infective hepatitis than in controls. These anomalies were observed during the first and third weeks after the onset of jaundice but had subsided by the sixth week.

Chromosomal aberrations did not appear to be related to the severity of infective hepatitis or to the sex or age of the patients.

The distribution of chromosomal abnormalities did not appear to be random; they were observed predominantly in the A₂ and B₄₋₅ series. Since no abnormalities were detected in the G-group chromosomes, no evidence in support of a relationship between infective hepatitis and Down's syndrome was obtained.

Numerical chromosomal aberrations were not observed, nor was any evidence obtained that mitotic activity of bone marrow cells is suppressed in patients with infective hepatitis.

Structural chromosomal aberrations have been described in patients with various viral diseases, such as measles (Aula, 1965; Nichols, Levan, Hall, and Östergren, 1962; Nichols and Levan, 1965), chickenpox (Aula, 1963, 1965; Massimo, Vianello, Dagna-Bricarelli, and Tortorolo, 1965), and mumps (Aula, 1965), as well as in subjects vaccinated against yellow fever (Harnden, 1964) or measles (Nichols, 1963).

Similar chromosomal changes have been shown to occur in tissue cultures infected by herpes simplex virus (Hamper and Ellison, 1961), simian virus 40 (Koprowski, Ponten, Jensen, Ravdin, Moorhead, and Saksela, 1962; Moorhead and Saksela, 1963), or oncogenic viruses (Shein and Enders, 1962).

It, therefore, appeared interesting to investigate whether chromosomal aberrations may be caused by infective hepatitis, especially in view of its probable epidemiological association with Down's syndrome (Stoller and Collmann, 1966).

Preliminary results from a small number of

children studied between the first and the 18th days after the onset of jaundice have already been reported (Matsaniotis, Kiossoglou, Maounis, and Anagnostakis, 1966).

Chromosomal findings in the bone-marrow cells of 27 children in the active phase of infective hepatitis and during remission are described.

Material and Methods

Twenty-seven children admitted to the Paediatric Clinic of Athens University from March to December 1966, in whom the diagnosis of infective hepatitis was well documented on both clinical and laboratory grounds, were included in the study.

Eleven patients were males and 16 females. Their ages ranged from 2 to 14 years. Their mental and sexual development was normal. None had been previously exposed to any form of x-ray radiation or drug therapy.

Chromosomal studies in direct bone-marrow preparations were performed during the icteric phase, one to six days following the onset of jaundice; during the phase of obvious clinical improvement, 15 to 20 days after the onset of jaundice, and during remission when the serum levels of bilirubin, and glutamic-oxaloacetic and glutamic-pyruvic transaminases approached normal limits.

Forty-four bone-marrow preparations were obtained, 21 during the first week, 16 during the third, and seven during the sixth week following the onset of jaundice.

The bone marrow was examined once in 15 patients, twice in seven, and three times in the remaining five patients.

Twenty-one patients with no viral disease, or haematological or neoplastic disorder, matched for age and sex, served as controls.

Chromosomal studies were carried out according to the method previously described (Kiosoglou, Mitus, and Dameshek, 1964). The combined Denver (1960) and Patau (1961) systems of nomenclature were used.

Coded material was used when reading patient and control slides.

Results

The number of patients examined, the time of examination, and the incidence of normal and abnormal findings are illustrated in detail in Table I.

	No. of Patients	No. of Bone-marrow Preparations	First Week	Third Week	Sixth Week
Once	15	15	11	4	—
Twice	5	10	5	5	—
Thrice	2	4	—	2	2
Thrice	5	15	5	5	5
Total	27	44	21	16	7
Chromosomal findings			A N	A N	A N
No. of bone-marrow preparations			12 9	12 4	1 6

Table I Findings in current series

A = abnormal, N = normal

The number of mitotic figures encountered in each preparation was about 20 in both patients and controls.

No. of Children	No. of Cells Examined	Number of Chromosomes				No. of Aneuploid Cells	No. of Cells with Structural Aberrations
		<46	46	>46	Polyploid		
21	430	26	387	2	15	28	5
Percentage		6.0	90.0	0.5	3.5	6.5	1.2

Table II Numerical and structural chromosomal aberrations in the control group

No. of Patients	No. of Cells Examined	Number of Chromosomes				No. of Aneuploid Cells
		<46	46	>46	Polyploid	
27	780	63	690	3	24	66
Percentage		8.0	88.5	0.4	3.1	8.4

Table III Numerical chromosomal aberrations in 27 patients

NUMERICAL CHROMOSOMAL ABERRATIONS
A total of 780 and 430 metaphase plates derived, respectively, from 27 patients and 21 controls, were counted and analysed on photomicrographs. The number of chromosomes ranged from 40 to 60 in both groups. The incidence of aneuploid cells was 8.4% in the patients and 6.5% in the controls. The number of polyploid cells was within the normal range, namely, 3.1% and 3.5% respectively (Tables II and III). The pattern was normal in all cells with 46 chromosomes. In the hypodiploid cells the loss of chromosomes was random.

STRUCTURAL CHROMOSOMAL ABERRATIONS

Isolated chromatid and isochromatid breaks and gaps were found in five out of 21 controls. The incidence of chromosomal aberrations was 1.2% (Table II).

Single or multiple chromatid and isochromatid breaks and gaps, acentric fragments, secondary constrictions, and minute chromosomes were demonstrated in 12 out of 21 patients during the first week, in 12 out of 16 patients during the third week, and in one out of seven patients during the sixth week, following the onset of jaundice (see Table I and Figures 1-3).

The number of affected cells and the incidence of aberrations during the first, third, and the sixth weeks following the onset of jaundice are shown in Table IV.

The incidence of cells showing structural abnormalities was 9.6% and 11.3%, respectively, during the first and third weeks, and 0.7% during the sixth week. The difference in the number of cells with structural aberrations between the patients and the controls was statistically highly significant ($p < 0.001$). Since many of the affected cells showed more than one aberration, in the group of patients the incidence of chromosomal abnormalities was 17.3% and 19.8% during the first and third weeks, respectively, ie, higher than that of cells with structural aberrations (9.6% and 11.3%). No structural changes were seen during the sixth week following the onset of jaundice.

In seven patients no chromosomal aberrations were detected (Table IV).



FIG. 1

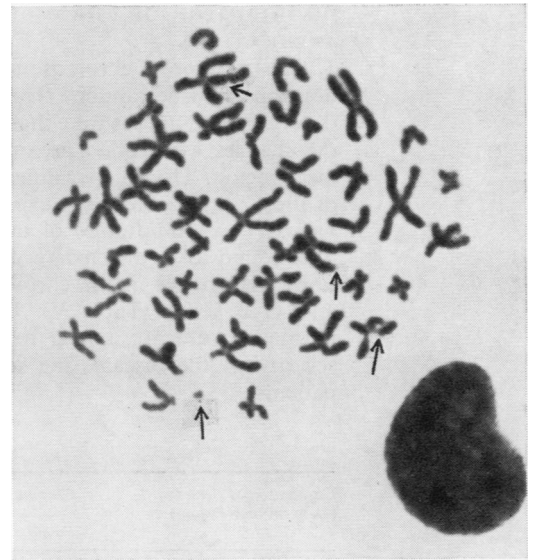


FIG. 3

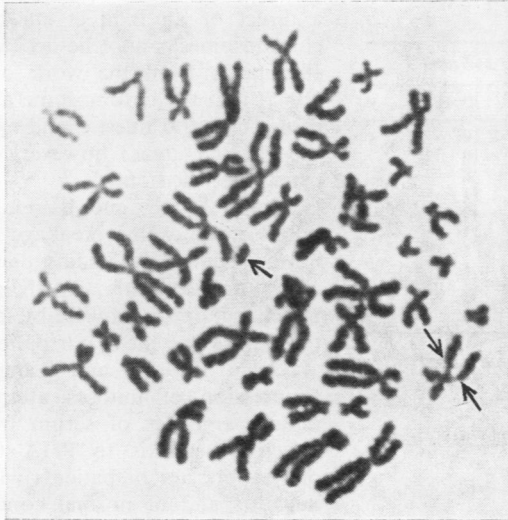


FIG. 2

Fig. 1 Bone-marrow metaphase plate containing 46 chromosomes. The arrows point to isochromatid breaks in a B_{4-5} chromosome ($\times 3,000$).

Fig. 2 Bone-marrow metaphase plate containing 46 chromosomes. The arrows point to chromatid break and isochromatid gaps in A and C-group chromosomes, respectively ($\times 3,000$).

Fig. 3 Bone-marrow metaphase plate containing 46 chromosomes plus one fragment. The arrows point to chromatin gaps, deletion, and fragment in A, B, and C-group chromosomes ($\times 2,500$).

Case No.	First Week			Third Week			Sixth Week		
	No. Cells Examined	No. Affected Cells	No. Chromosomes Aberrant	No. Cells Examined	No. Affected Cells	No. Chromosomes Aberrant	No. Cells Examined	No. Affected Cells	No. Chromosomes Aberrant
1	15	6	13	—	—	—	—	—	—
2	13	2	7	—	—	—	—	—	—
3	7	0	0	—	—	—	—	—	—
4	32	1	1	—	—	—	—	—	—
5	27	3	7	—	—	—	—	—	—
6	2	0	0	—	—	—	—	—	—
7	10	0	0	—	—	—	—	—	—
8	—	—	—	25	1	1	—	—	—
9	8	4	7	—	—	—	—	—	—
10	—	—	—	15	2	4	—	—	—
11	—	—	—	18	1	3	—	—	—
12	16	1	1	—	—	—	—	—	—
13	—	—	—	15	3	6	—	—	—
14	15	0	0	3	2	7	—	—	—
15	29	0	0	21	2	4	—	—	—
16	3	2	5	—	—	—	—	—	—
17	10	2	3	22	3	6	22	0	0
18	17	2	3	24	3	5	—	—	—
19	—	—	—	14	0	0	23	0	0
20	—	—	—	11	4	5	14	0	0
21	19	0	0	10	0	0	20	0	0
22	21	0	0	20	0	0	26	0	0
23	30	4	5	15	3	4	15	0	0
24	20	4	5	27	5	7	25	1	1
25	20	0	0	17	0	0	—	—	—
26	10	0	0	26	3	4	—	—	—
27	28	3	4	—	—	—	—	—	—
	352	34	61	283	32	56	145	1	1
Total Percentage		9.6	17.3		11.3	19.8		0.7	0.7

Table IV Chromosomal aberrations in 27 patients with infective hepatitis after onset of jaundice

DISTRIBUTION OF CHROMOSOMAL ABERRATIONS

The distribution of chromosomal aberrations did not appear to be random. The chromosomes of the A₂ and B₄₋₅ series were those mainly affected; the changes were found proximal to the centromere region. The abnormalities in chromosomes of the C series were those expected on a chance basis. The chromosomes of the D and E series were found to be affected very little. No aberrations were noted in the chromosomes of the F and G-Y series (Table V). Chromosomal abnormalities did not appear to be related to the severity of the disease, the sex, or age of the patients.

Chromosomal Series (Denver ¹ and Patau ²)	Chromosomal Aberrations	
	Found	Expected
A { 1	11	10
2	25	10
3	4	8
B 4-5	30	13
C 6-X-12	35	42
D 13-15	4	11
E 16-18	3	8
F 19-20	0	5
G 21-22	0	4
Y	0	2

Table V Distribution of chromosomal aberrations on the various series in 27 patients with infective hepatitis

¹Lancet, 1, 1063 (1960).

²Lancet, 1, 933 (1961).

Discussion

The results of this study showed that chromosomal aberrations can be found in a considerable proportion of patients with infective hepatitis between the first and the third weeks following the onset of jaundice.

Similar chromosomal changes have been reported in neoplastic disorders (Kiosoglou *et al.*, 1965; Sandberg, Ishihara, Kikuchi, and Crosswhite, 1964). The presence of similar chromosomal aberrations in viral diseases and neoplasias raised a growing interest about the role of viruses in the development of carcinogenesis (Nichols, 1963; Porter, 1963); it was postulated that chromosomal breakage might represent the primary gene mutation, presumably leading to the development of neoplasia.

Whether the chromosomal aberrations demonstrated in this study may be associated in some way with the postulated epidemiological relationship between infective hepatitis and Down's syndrome (Stoller and Collmann, 1966) cannot be decided yet. In any event, no numerical or structural aberrations in the G₂₁₋₂₂ series which would support such a relationship between infective hepatitis and Down's syndrome were detected in this study.

The disappearance of the chromosomal aberrations detected during the first and the third weeks following the onset of jaundice may be attributed to destruction and elimination from the circulation of cells with imbalanced genetic material because of their inability to replicate.

Why some patients did not demonstrate any chromosomal aberrations is difficult to decide; however, the presence of chromatid and isochromatid breaks and gaps in various viral diseases, including infective hepatitis, suggests that breakage may be connected with the interaction between cells and viruses.

Aberrations may result from disordered DNA synthesis due to blockage in the formation of one of several DNA precursors. Whether this is a direct or an indirect effect of the virus on chromosomes cannot be decided at the moment. It appears from the work of Rapp and Hsu (1965) that the chromosome aberrations are not caused by viral nucleic acid synthesis at or near the affected areas; however, other viral effects cannot be ruled out.

El-Alfi, Smith, and Biesele (1965) have produced chromosome breakage in cell cultures of normal persons by adding plasma from patients with viral hepatitis; in their cases, however, the changes induced were by far less pronounced than those present in our patients.

Recently Mella and Lang (1967) reported suppression of mitoses after the addition of minute amounts of serum from patients with infective hepatitis to PHA-stimulated cultures of lymphocytes of patients with active infective hepatitis and of normal controls. Serum from convalescing patients did not show this suppressive effect, but it did cause structural chromosomal aberrations, which were attributed to cell injury or interference with cell replication by the virus of hepatitis.

On the other hand, Tobias, Safran, and Schaffner (1967) reported more than 80% transformation into large lymphoid cells in PHA-stimulated cultures of lymphocytes from patients with various liver diseases, including viral hepatitis, indicating that the cells were capable of responding promptly.

In our study suppression of mitotic activity of bone-marrow cells was not observed, since mitotic figures in numbers comparable to those observed in controls were found at all stages of infective hepatitis.

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