Viruses and Their Effect on Human Chromosomes

Yavuz Renda, M. D.*

The structural changes of human chromosomes produced by viruses have been previously investigated.[1] [2] Most of the research found in the literature is on human viruses such as measles, chickenpox and, to a lesser degree, mumps. There are also various reports about oncogenic viruses,[3] [4] [5] [6] toxic agents,[7] [8] radiation[9] and their effects on the chromosomes. The abnormalities caused can be divided into three groups: 1) constrictions, 2) breaks and 3) aneuploidy and hyperploidy.

De la Chapelle[10] showed secondary constrictions on the normal karyotypes, especially on the 6 - X - 12 group, and proposed that these constrictions are the normal variations. Nichols, Levan, Hall and Ostergen[1] and Nichols[11] have studied the peripheral leukocyte cultures of measles patients and have seen chromatid breaks from 32 to 70 per cent. On the other hand, Tanzer et al[12] published a report with the opposite results. Others[13] have worked on peripheral blood leukocytes *in vitro* and have shown a mitotic inhibition when the leukocytes were incubated with viruses.

The purpose of our study is to investigate the effect of nononcogenic viruses on human chromosomes, to demonstrate the structural changes and to compare our results with the findings of other authors.

Materials and Methods

Cases were selected from patients with infectious diseases in the Out - Patient Department. All the cases which were diagnosed as measles, chickenpox, mumps or infectious hepatitis were included in the study with the exception of four groups : 1) patients who were on medication of any kind, 2) patients who were receiving radiation therapy, 3) patients who also had other types of disorders and 4) patients whose cases were doubtful.

From Hacettepe University Faculty of Medicine, Department of Pediatrics, Ankara.

* Assistant Professor of Pediatrics.

Seven chickenpox, eight measles, nine mumps and 17 infectious hepatitis cases were selected for this investigation. Only the first three conditions will be discussed in this paper; the fourth will be the subject of another report.

The control group was selected from among patients who had been admitted to the chromosome laboratory for other reasons. There were 11 nonviral diseases, five Down syndrome (21 - Trisomy), five parents of Mongoloids and 18 healthy persons chosen from the hospital personnel, a total of 39.

In the peripheral blood leukocyte culture, Moorhead et al's [14] modified technique was used and the Denver classification was accepted for the analysis of karyotypes. ADOX RT 14, 35 mm black and white film was used for the microphotographs.

Results

Among the viral cases, a total of 545 cells at the metaphase were counted. All cases were found to have normal karyotypes. Cells with polyploidy were not included in the study due to their scarcity.

Among the control patients, 719 cells at the metaphase were counted. Except for the 21 - Trisomy cases, all the other patients also had normal karyotypes.

Distribution of individual chromosome numbers of both groups is illustrated in Table 1 : the nonviral disease group showed the highest percentage of normal diploid numbers (86.8); the healthy subjects followed with 82 per cent; and the measles cases displayed the lowest percentage (65.76).

Statistically there was a significant correlation between the chromosome number in cells of patients with viral diseases and controls ($X^2 = 79.441$). On the other hand, there was no significant correlation between the chromosome number in cells of the nonviral cases and normal individuals.

Table 1 also gives the structural anomalies of the chromosomes produced by the viruses. The frequency of breaks showed a significant increase ($X^2 = 44.338$) among the viral cases when compared with the control group. The results were the same for constrictions ($X^2 = 17.781$). Other anomalies such as dicentric chromosomes, tricentric chromosomes or ring formations are not included in the study since their number was not significant.

The distribution of abnormalities according to the day of sampling (one to three days, four to seven days and over eight

TABLE 1

DISTRIBUTION OF CHROMOSOME NUMBERS AND STRUCTURAL ABNORMALITIES OBSERVED IN VIRAL DISEASES AND CONTROLS

Cases	Total cells counted	Number of cells						Breaks		Constrictions	
		46	%	<46	%	>46	%	Number	%	Number	%
Chickenpox	160	122	76.25	20	12.50	18	11.25	14	8.8	15	9.3
Measles	184	121	65.76	37	20.21	26	14.03	31	16.9	12	6.5
Mumps	201	141	70.15	46	22.77	14	7.08	22	11.0	19	8.9
Normal	340	279	82.05	25	7.35	36	10.60	16	4.7	15	4.4
21 - Trisomy	89	61	68.53	17	19.10	11	12.37	3	3.4	5	5.6
Parents of Mongoloids	70	53	75.70	13	18.57	4	6.73	3	4.3	5	7.1
Nonviral cases	220	191	86.80	17	7.72	12	5.48	6	2.8	11	5.0

days) was also investigated and no significant correlation was noted.

Discussion

Structural changes of the chromosomes are common under the influence of both extrinsic and intrinsic factors. Breaks, constrictions of chromatids and aneuploidy or hyperploidy are probably the most frequent type of abnormality which can be visualized microscopically.

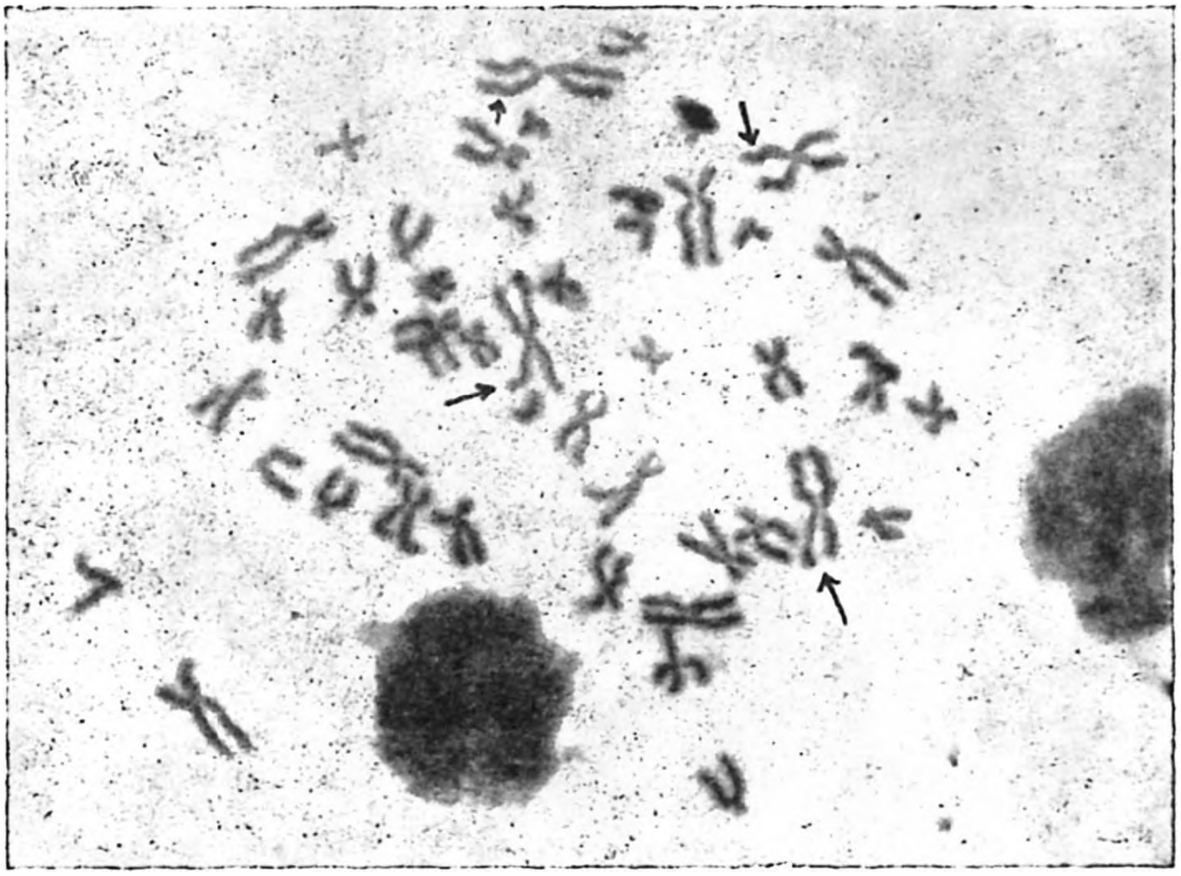
Constrictions can be classified as primary and secondary : primary constrictions are located over the centromer, while secondary constrictions are found anywhere along the chromatids. Satellites of the acrocentric chromosomes are secondary constrictions which are formed normally. These satellites function in the development of the cell nucleus. [15] [16] Besides this natural occurrence, other conditions, such as preparation of the fixative [17] and keeping the cells in a medium low in calcium ion, [18] increase the amount of secondary constrictions. Autoradiographic investigation revealed that the basic structure of the DNA molecule on the constricted region is somewhat different from the rest of the chromatid (Figures 1 and 2). [19]

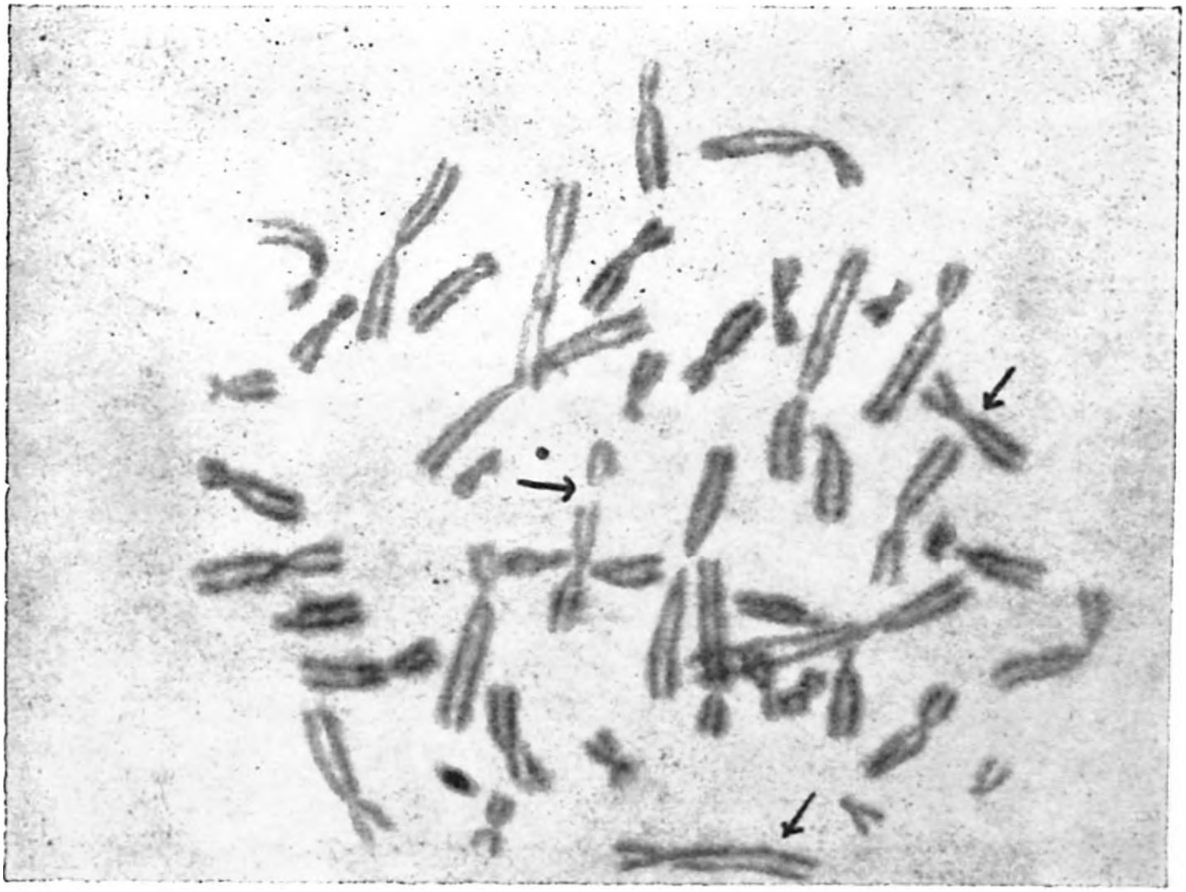
Breaks are the complete separation and deletion of the chromatids. Ostergren and Wakonig [20] have used the term «delayed isolocus breaking» when there is a break on one chromatid and a constriction on the same locus of the isochromatid. Achromatic spaces on a chromatid can also be regarded as breaks (Figures 1 and 2).

The term aneuploidy is used when the human diploid chromosome number is less than 46; when this number is over 46, the term used is hyperploidy. If hyperploidy shows a regular tendency toward multiplication of diploidy, it is called polyploidy (Figure 3).

The incidence of breaks is reported by different authors [1] [9] [10] to be around five per cent among normal individuals. Factors like previous radiation, medication and some intranuclear biochemical changes can play an important role in increasing this amount. Preparation of slides and different techniques can also affect the results.

Nichols et al [1] [11] [13] studied children with measles and obtained a percentage of breaks from 32 to 70. They have also noticed pulverization of chromosomes. In their study of children who had been





Figures 1 and 2. Karyotypes of two leukocytes at metaphase. Arrows indicate breaks and constrictions on several chromosomes.

Figure 3. Karyotype of a leukocyte showing polyploidy.

vaccinated for measles, Nichols and Levan [21] found normal karyotypes and did not see any excess breakage or constriction. Aula [2] studied the peripheric leukocytes of chickenpox patients and demonstrated the breakage of chromatids of these patients to be as high as 28 per cent. Breaks in his control group were only 4.3 per cent.

Benyesh - Melnick et al, [22] developing tissue culture by using human embryo lung and adding herpes zoster viruses in the medium, noted breaks of chromosomes from 25 to 45 per cent. El Alfi et al [23] published a similar report concerning five cases with infectious hepatitis.

Our results on viral cases showed the incidence of breaks to be from 8.8 to 16.9 per cent which is much lower than the figures found in the literature. The incidence of breaks on our control cases, however, showed a parallelism to the ones in the literature. Nevertheless, our viral cases were found to have statistically a significantly higher amount of breaks and constrictions.

It is possible that different techniques in sampling the blood and running the tests could be the cause of the low percentage

of anomalies in our study; however, the day of sampling did not prove to be an important factor in the occurrence of abnormalities.

The tendency toward increased aneuploidy, breaks and constrictions is a clear sign of the harmful effects of viruses on chromosomes. The exact ways by which these changes are brought about are still obscure. Kihlman et al [8] suggested that a disturbance of the DNA synthesis of chromosomes could cause abnormalities. The same picture is frequently caused by other factors such as radiation, oncogenic viruses and toxic agents.

Animal viruses are also used to investigate this phenomenon. Tsuchida and Rich, [34] Chardonnet and Pruniéras [25] and Pruniéras et al [26] used Friend and Rauscher SV 40 virus on hamsters and showed several constrictions and breaks. Several others obtained similar results. [5, 27, 31]

The nature of the breaks and constrictions produced by DNA analogues such as desoxyadenosine and cytosine arabinoside is not different from the breaks of the viral type. [7, 8] Some authors have proposed that these breaks occurred because of inhibition of DNA synthesis by these agents; [32] others have opposed this idea.

This study confirms that nononcogenic viruses have the same destructive capacity on human chromosomes and genetic material as oncogenic viruses and toxic agents. This damage to the organism is probably temporary and can occur during viremia. Long-term studies will provide the answer to this question. It has been shown that agents like radiation could cause chromosomal disorders for as long as twenty years. [9, 35] At present we can only conclude that chromosome anomalies produced by various viruses can be one of the early stages of the cytopathic effect of the viruses which kill the cell.

Summary

This investigation was performed in order to study the effect of nononcogenic viruses on human chromosomes. Measles, chickenpox and mumps cases were selected and peripheral leukocyte cultures were obtained for chromosomal analyses. These cases showed significantly higher amounts of structural anomalies than the control group. The results and the pathogenesis of this phenomenon are discussed.

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