

Chromosome Abnormalities in Peripheral Blood Cells of Post-Polio Patients in South Indian Population

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Abstract

A number of viruses have been shown to be capable of producing abnormalities of the metaphase chromosomes in circulating lymphocytes during some natural infections in man, i.e., measles virus, chicken pox and mumps viruses and hepatitis virus. However this cataloguing of viruses with a demonstrated capacity for direct or indirect production of effects on metaphase chromosomes is most certainly incomplete. Thus even the immediate effects of a high number of viral genomes, important one being poliovirus on chromosome is still wanting. Further follow up studies on the delayed late effects of viral genome on the human leukocyte chromosomes was much less in literature. Hence to fulfil the lacunae a study is undertaken to investigate mainly the delayed effects of polio virus on human chromosomes in post polio syndrome. The control group consisted of 30 healthy subjects for chromosomal aberration analysis. All the control subjects were matched for age and with no history of Polio. The study group consisted of 30 Polio patients for analysis of cytogenetic aberration. The details of sex, age at onset, severity of disease, exposure to chemicals, smoking and consanguinity is presented for the 30 PPS individuals. In this study the frequency of chromosome aberrations in PPS showed a significant increase as compared with the controls.

1. Introduction

Poliovirus (PV), the etiological agent of paralytic poliomyelitis, belongs to the *Picornaviridae* family. It causes paralysis due to destruction of motor neurons, a consequence of PV replication. Since 1961, when the first report appeared on chromosome breakage following virus infection with Herpes simplex virus of cells in culture [Hamper, 1961, 1967]. Further, a number of viruses have been shown to be capable of producing abnormalities of the metaphase chromosomes in circulating lymphocytes during some natural infections in man, i.e., measles virus, chicken pox, mumps virus and hepatitis virus [Aula, 1963, 1965; Nichols, 1965, 1969]. However this cataloguing of viruses with a demonstrated capacity for direct and indirect production of effects on metaphase chromosomes is most certainly incomplete. Further the variety of possible combinations of host cells with a particular virus has resulted in numerous reports, of which only a few represent independent assessments of the same cell-virus combinations. Therefore mutually supporting studies should not be neglected.

Convincing hypothesis for the agreement of the DNA within the mammalian metaphase chromosome and the complexities of relations between an agent's primary effect and secondary ones mediated through the cells metabolism upon

both chromosome and mitotic apparatus are urgently needed. The fact that so many viruses as well as other agents induce both breakage and disruption of normal function of mitosis has no satisfactory explanation, except in the surmise that it could be caused by point mutation which controls cell division. The immediate effects of a high number of viral genomes, important one being poliovirus, on chromosome still wanting. Further, follow up studies on the delayed effects of viral genome on the human leukocyte chromosomes much less in literature. Hence to fulfill the lacunae a study is undertaken to investigate mainly the delayed effects of polio virus on human chromosomes in Post Polio Syndrome (PPS). PPS is a chronic condition of the Central Nervous System (CNS). PPS became an important medical and epidemiological problem after the epidemics that occurred worldwide in 1940s and 1950s, until vaccination became available [Ramlow et al., 1992]. The estimated number of persons affected by PPS is around 20 million worldwide because of high number of polio survivors [Rekand et al., 2004]. The pathogenesis of PPS is not completely understood [Ragonese et al., 2005; Munsat, 1991].

PPS tends to diagnose anywhere from 10 to 40 years after the initial polio infection, however not everyone who has had polio will develop PPS

[Nordgren et al., 1997]. Signs and symptoms vary in duration and severity and they can include symptoms like – fatigue, muscle weakness, joint and muscle pain, muscle twitching, difficulty sleeping, gastrointestinal problems, breathing – swallowing difficulties, intolerance to cold. They can lead to the severe condition like muscle atrophy, osteoarthritis, osteoporosis, skeletal deformities and nerve entrapments. During the period between 1920-1970 biochemical and cytogenetic techniques were developed for human syndrome analysis; however no reports so far on the cytogenetic study for PPS are available. Therefore, in this an attempt has been made for the first time to make use of the cytogenetic technique to analyze the frequency of chromosomal aberrations.

2. Materials and Methods

This study includes 30 cases of Post polio syndrome. The control group consisted of 30 unrelated healthy donors. The controls were not affected by any diseases. Information regarding parental age, parental consanguinity, birth order of the proband, familial incidence of the anomaly, if any, reproductive history of the mother, and other

associated disorders of the probands were recorded for future genetic studies.

Leucocyte Culture method

Blood samples were obtained with informed consent of the patients and parents. Heparinised blood was collected after venous puncture from the patients. Chromosome preparations from the lymphocyte cultures for karyotype analysis were made according to the modified method of Hungerford (1965).

Chromosome preparation method

For each patient and control, three culture vials (each containing 0.6 ml of blood, 6 ml of culture medium (RPMI 1640, Invitrogen), 1.2 ml of Fetal Bovine Serum (FBS, Himedia) and 0.4 ml of Phytohaemagglutinin (PHA, Invitrogen) were added. The metaphase were arrested with the addition of colchicines (0.025%, Sigma) at 72 h. Cells were subjected to hypotonic treatment (0.075M KCl, SD Fine) and fixed in methanol : acetic acid (3:1), slides were made and G – banded.

Frequency of chromosome breaks analyzed

The frequency of chromosome breaks analyzed in PPS is presented in Table-1.

Table 1: Frequency of chromosomal aberrations in Control & PPS individuals

Subjects	No. of Metaphases Analysed	No. of Breaks Observed (\pm)		Total Aberrant Cells (\pm)
		Chromosomal Breaks	Chromatid Breaks	
Control (n=30)	1500	18 (3.17 \pm 1.2)	13 (2.29 \pm 0.86)	63 (11.11 \pm 4.2)
Post Polio Patients (n=30)	1610	68 (12.0 \pm 4.22)*	116 (20.47 \pm 7.20)*	564 (99.53 \pm 35.03)*

* Significant at $\alpha=0.05$ ($p<0.0001$)

Estimation of the frequency and the pattern of chromosomal break points on all 23 types of G – banded chromosomes

For each patient and control, 50 G – banded well spread metaphase were scored by direct microscopic analysis and photography whenever needed. The frequency and distribution pattern of chromosomal break points associated with all types of structural abnormalities, such as chromosome breaks, deletions, rings, isochromosomal breaks and dicentrics for every type of chromosome involved were recorded. Few structural aberrations and rings were not included in this study.

Statistical analysis

Statistical analysis has been done using GraphPadPrism Version 5.1 (Graph Pad Software Incorporation, San Diego, CA). Difference among groups was first analyzed by a non-parametric Mann-Whitney test. Differences were considered significant at $P < 0.0001$.

3. Results

The detailed analysis of this study is presented.

Frequency of chromosome aberrations analyzed in controls

30 controls, age matched, with no smoking habits and no disease histories were identified and the frequency of chromosome aberrations analyzed and presented in Table: 1. The frequency of total abnormal cells 63 out of 1500 metaphases (11.11 ± 4.2) was significantly higher than the frequency of breaks i.e. chromosomal breaks-18 (3.17 ± 1.20) and chromatid breaks-13 (2.29 ± 0.86) analyzed.

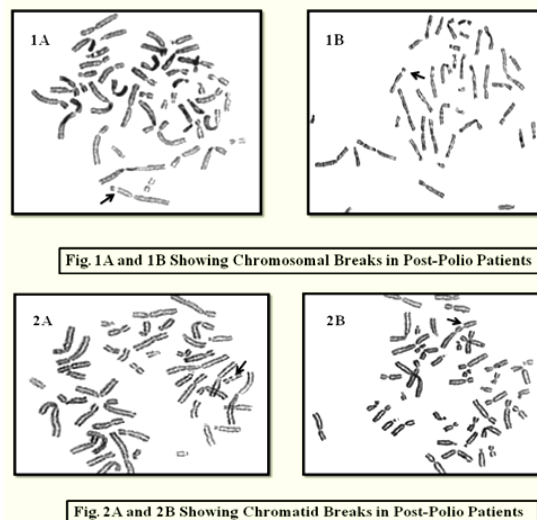
Frequency of chromosome aberrations analyzed in PPS individuals

The different types of chromosome aberrations observed in PPS are presented in plates attached.

Frequency of chromosome breaks analyzed

The frequency of chromosome breaks analyzed in PPS is presented in Table-1. In the frequency of cells with chromosome breaks analyzed in PPS i.e., 184 i.e. chromosomal breaks-68 (12.0 ± 4.22) as shown in fig. 1A and 1B and chromatid breaks-116 (20.47 ± 7.20) shown in fig. 1B and 2B out of 1610 cells, a significant increase was observed i.e., (32.47 ± 11.42) as compared with that of controls i.e., (5.46 ± 2.06).

Thus, in this study the frequency of chromosomal aberrations in PPS showed a significant increase as compared with the controls.



4. Discussion

Several studies reported an association between disease severity, age at onset of poliomyelitis and PPS [Dalakas, 1995; Hsu, 1961]. However Ragonese *et al*, 2005 observed that there is no association between polio severity and PPS in his study. Our results also show no association between age at onset, clinical severity of disease in PPS. The contribution of smoking to the increased pathogenesis of PPS is much debated [Burk and James, 2000]. However in our study none of the PPS individuals had smoking habit or were exposed to any genotoxic chemicals. The viral induced chromosome aberrations in human leukocytes were observed [Stich and Yohn, 1970]. There are several reports of chromosome breakage *in* leukocytes cultured from hepatitis patients [Aya, 1967; El Alfi, 1965; Emerit and Emerit, 1972]. There are also reports of chromosome damage in bone marrow cells [Emerit and Emerit, 1972]. There are probably

only reports of viral induced chromosome damage *in vivo* in man.

Chromosome breakage in lymphocytes of patients with congenital rubella has been reported [Kuroki *et al*, 1966; Aya, 1967; Nusbacher, 1967] although several authors have failed to find out any evidence of damage [Mellman, 1965]. In cells cultured from fetuses aborted because of maternal rubella infection [Chang *et al*, 1966], and in human cells infected with rubella virus *in vitro* [Chang *et al*, 1966], an increased incidence of breakage was reported in cases where an adequate number of cells were studied. In study of over 2000 metaphases, [Chang *et al*, 1966] found no evidence of chromosomal rearrangements. The kinds of chromosome aberrations appearing at late effect of virus infection are different from those occurring earlier in that the main features are chromosome and chromatid breaks and exchanges, aneuploidy and a tendency to polyploidy. Chromosome breaks and gaps do occur, but they are normally repaired. In our result we observed significantly increased

chromosome aberrations in PPS as compared with that of controls. Chromosome breakage in human cell strains and in an established cell line has been reported to occur within 6 hours of infection with high multiplicity of poliovirus [Bartch et al., 1967]. Again there was not evidence of chromosome rearrangements. However no association between the clinical severity of PPS and the frequency of chromosome aberrations could be made. A thorough study with a large sample size will prove if the severity of PPS is associated with the frequency of chromosome aberrations. PPS occurred several years after the attack of poliovirus. Hence the chromosomal aberrations occurred in that are not produced immediately after the viral attack. The types of aberration observed are chromosome breaks and mainly chromatid breaks are typical of mitotic chromosome aberrations. These aberrations can be compared with the aberrations observed in chromosome instability syndromes. Hence it will be interesting if we follow PPS prospectively to know that, will PPS lead to cancer just like instability syndromes (Ataxia telangiectasia and Fanconi anemia) prone to cancer. That study would show the viral origin of cancer even several years after the viral infection.

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