Vet.Med.J.,Giza. Vol.43,No 4. (1995): 415-420.

CHROMOSOMAL STUDIES IN BUFFALO GREEN MONKEY CELLS INFECTED WITH POLIOVIRUS TYPE 3.

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SUMMARY

Metaphase chromosomes were prepared from Buffalo green monkey kidney cells. The diploid chromosome number was 60. The autosomal chromosomes consisted of 36 meta-or submeta-centric and 22 were acrocentric. The sex chromosomes were submetacentric in X and a small subacrocentric in Y. Chromosome aberrations were studied as a result of poliovirus type 3 infection, and consisted of chromatid gaps and breaks, chromosomal gaps and breaks, deletions, centromeric attenuations, peridiploidy and polyploidy. The abnormalities were more frequent in infected cells than in normal ones. All chromosomal aberrations except chromatid gaps, deletions, centromeric attenuations and peridiploidy were significantly higher in infected cells. The results showed that poliovirus type 3 had mutagenic effect on the chromosomes.

INTRODUCTION

Poliomyelitis is an acute infectious disease of world-wide distribution, that may spread in endemic form. In Egypt there was no special attention to poliomyelitis before the Second World War. At that time the disease was observed among the British Troops stationed in Egypt. Since 1951-1952, There was more interest, better diagnosis and better reporting of cases (Imam, 1975). Cytogenetical analysis for patients adds an important role to clinical diagnosis. This procedure shows the effect of the pliovirus on the chromosomes. Structural or numerical aberrations of chromosomes can occur as a result of exposure of animal cells to unnatural agents including viruses infection (Bongso and Basrur, 1976. Cribiu and Popescu, 1980, Brusick, 1980 Pai, 1985). Viruses infection could induce mitotic disturbances leading to polyploidy or peridiploidy (Cribiu and Popescu, 1980). Many authors described the bad mutagenic effects of the viruses in mammalian cells such as tumors (Awano and Amano, 1971), hepatitis B (Tokino et al., 1991) and Simian 40 virus (Lehman and Robinson, 1978, Lehman and Trevor, 1979 and Ray et al., 1992). In the present study the karyotype of Buffalo green monkey was studied for chromosomal abnormalities as a result of poliovirus type 3 (Saukt strain) infection..

MATERIALS AND METHODS

1- Cells and Virus:

Buffalo green monkey cells (BGM) were grown in 25 cm^2 tissue culture flasks . Minimum essential medium was used with 10% foetal calf serum, 1% antibiotic-antimycotic mixture (10.000 IU penicillin, 10,000 ug streptomycin and 25 ug fungizone / ml.) and pH was adjusted at 7.2-7.4

with 7.5% sodium bicarbonate solution. Cells wre infected with poliovirus type 3 (Saukett strain) for 12 hr at multiplicity of infection equal 0.1.

2- Chromosome preparations:

The metaphase chromosomes were prepared according to Cribiu and Popescu, (1980), by adding 0.3 ml of 0.05% colchicine solution per each 25 cm^2 flask for 1-1.5 hr prior to harvesting of the cells. Cells were dissociated with trypsin solution (0.15% trypsin 1:250, 0.04 versene). Cells were colected and treated with hypotonic solution (0.075 M potassium chloride) for 25-30 min, then fixed in 3:1 methanol acetic acid and dropped on slides covered with ethanol 70% at 4°C. Slides were stained with Giemsa stain and then examined for chromosomal analysis. For chromosome studies, 450 metaphase spreads in control cells were analyzed , while in infect cells 558 metaphases were examined . The resul were statistically analyzed using the Chi-squa test.

RESULTS

The karyotype of the Buffalo green monke derived African green monkey was prepared this study of normal cultures of kidney cells vitro. The diploid chromosome number (2n) wa 60 (Fig.1). The autosomal chromosomes consiste of 36 meta-or submetacentric and 22 wer acrocentric. The sex chromosomes wer submetacentric in X and a small subacrocentric i Y. In the present study, structural and numerica aberrations of the chromosomes were observe Structural aberrations consisted of chromatid

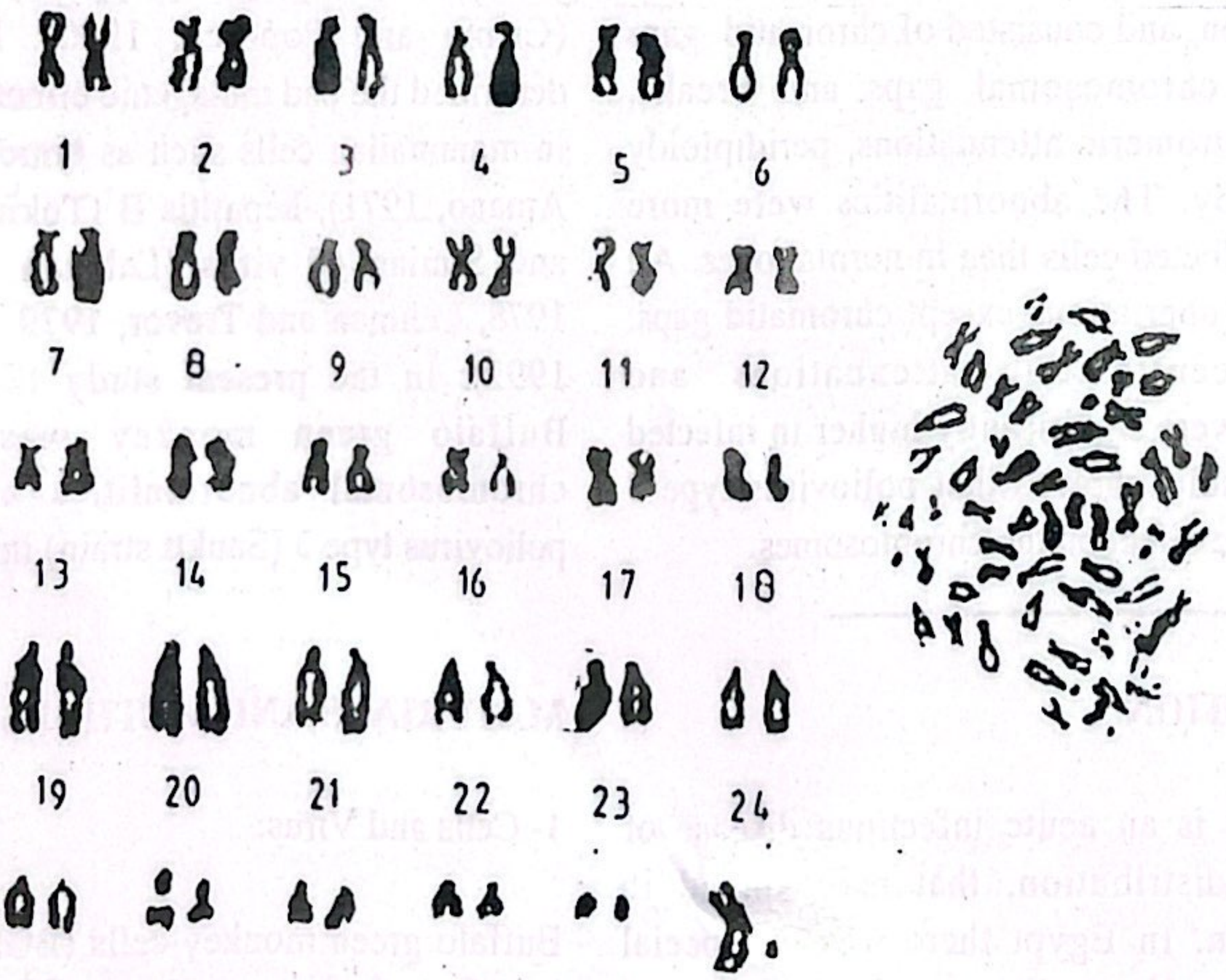

Fig. (1): Metaphase spread and karyotype of normal cells of Buffalo Green Monkey male *(Cercopithecus aethiops).*

Table (1) Percentages and Chi-square values of chromosome aberrations in Buffalo Green monkey cells normal and infected with poliovirus III (Saukett strain) .

Group	Total Metaphase spread	Structural aberrations							Numerical aberrations	
		chromatid gaps	chromatid breaks	chromos-omal gaps	chromoso-mal breaks	deletions	Centrom-eric atte-nuations	T.struc-tural	peridiploidy	polyploidy
Percentage (%) of normal cells	450	0.2	0.0	0.0	0.0	0.0	1.1	1.3	2.0	4.9
Percentage (%) of infected cells	558	1.25	1.79	1.79	2.33	0.18	1.25	8.6	2.7	24.0
Chi-square values between normal and infected cells		3.4	** 8.2	** 8.2	** 10.6	0.81	0.04	** 25.96	0.51	** 69.7

* Significant at level 0.01 .

Fig. (2): Metaphase spread of *Cercopithecus aethiops* showing polyploidy.

gaps, chromatid breaks, chromosomal gaps and breaks, centromeric attenuations, deletions and fragments, the latter was scored as chromatid breaks for statistical analysis. Numerical changes were polyploidy (Fig.2) and peridiploidy, the latter including cells with 59,61,58 and 62 chromosomes. Results of the cytogenetical examination are listed in Table (1). Chromosomal anomalies were few or absent in control cultures while they were more frequent in infected ones. Chi-square analysis of the structural differences between normal and infected cultures showed that the differences of the frequencies for each of chromatid breaks, and chromosomal gaps and breaks were highly significant in infected cells than in control ones. The differences of the frequencies for each of chromatid gaps , deletions and centromeric attenuations were not significant. Differences of numerical aberrations (polypliody) was highly significant in the infected cells. The differences in peridiploidy frequency between normal and infected cultures was not significant.

DISCUSSION

Buffalo green monkey derived African green monkey species *(Cercopithecus aethiops)* is placed in the genus Cercopithecus (which contains 12 species), family *Cercopithecidae, order,* Primates (Walker et al., 1964). The chromosome number within the genus Cercopithecus varies from 54 to 72 (Chiarelli, 1969). The diploid chromosome number of African green monkey is 60 (Markarian et al., 1969; Yanagisana, 1971 and Stock and Hsu 1973). The karyotype of the Buffalo green monkey examined in the present study was similar with that reported for the African green monkey in the literature and consisted of 2n=60

chromosomes. When BGM cells were infected with poliovirus , a typical growth curve for infectious virus was seen with eclipse of approximately 90% of input virus at 0- 2hr, a lag phase of 2-4 hr, and a final yield at 12-15 hr postinfections (Anderson et al., 1988). So in the present study cytogenetical analysis was studied after 12 hr to observe the chromosomal damages which were more frequent and highly significnat in infected cells with comparison to normal ones. These types of chromosome aberrations included, chromatid breaks, chromosomal gaps and breaks, and polyploidy. The samilirities of chromosomal aberrations were observed in other mammalian cells by many types of viruses. Polyploidy occurred by Simian virus (SV40) (Lehman, 1974, Ray et al., 1992) or by tumor virus (Awano and Amano, 1971). Chromatid breaks were induced by SV40 (Lehman, 1974), while chromosome breaks resulted by hepatitis B virus (Simon et al., 1991). Chromatid deletions occurred by vaccinia virus (Mikhailove et al., 1974) or by SV40 (Lehman and Trevor, 1979) or by hepatitis B virus (Rogler et al., 1985, Tokino et al., 1991). In the present study , the high incidence of chromosome aberrations occurred in infected cells showed that poliovirus had mutagenic effect on the chromosomes.

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