STUDY OF EFFECT OF COLCHICINE EXPOSURE ON LENGTH OF CHROMOSOME DURING MITOSIS

Preeti R. Koyani, S.S. Saiyad
Genetic /Research laboratory, Department of Anatomy,
Pandit Dindayal Upadhyay Medical College, Rajkot, Gujarat.

ABSTRACT

In routine karyotyping procedure from lymphocyte culture (blood culture) cells are exposed to colchicine to arrest cell division at metaphase to visualise chromosomes. Routinely duration of exposure to colchicine varies from 1 to 2 hours. To get better quality of chromosomes both the concentration and period of exposure to colchicine can be altered.

While standardization of departmental Genetic/Research laboratory trial different period of exposure time of colchicine was done to get longer chromosomes with splitted chromosomes.

It was observed it was marked that by shortening the exposure period of colchicine longer and better quality of chromosomes can be obtained whereas longer exposure period of colchicine gives condensed chromosomes.

Key word : Chromosomes, colchicine, condensation

INTRODUCTION

From cytogenetic stand point it has been noted that shortening of the chromosomes, chromatin condensation coincides with a remarkable prolongation of DNA synthetic phase[1]. The packaging of chromatin is flexible; it changes during eukaryotic cell cycle. At the time of division, the genetic material becomes even more tightly packaged and individual chromosomes become recognizable[2]. The precise segregation of replicated chromosomes to daughter cells during mitosis depends on formation of bipolar spindle composed primarily of microtubules (MT)[3] .Since MTs is highly dynamic structure whose spatial organization is critical for proper spindle function. Physical &chemical agents that interfere with MT behaviour invariably disrupt mitosis[3].

Colchicine inhibits microtubule polymerization by binding to tubulin, one of the main constituent of microtubule it specifically inhibits the development of spindles or destroys those already present, so the cell cannot move its chromosomes around and the cell may end up copying some or all of the chromosomes and cannot parcel them out into new cells and so it never divides.[4]

Correspondence
Dr. S. S. Saiyad
Professor and Head of Department,
Anatomy, PDU Medical College,
Rajkot

Human mitotic metaphase plates are routinely prepared by employing the modified method of Moorhead et al.(1960)[5]. Study was aimed to see the effect of various exposure periods of colchicine (concentration of colchicine was kept same in each exposure.) on lymphocyte culture during mitosis by measuring length of longest chromosome in metaphase spread.

MATERIAL AND METHOD

Peripheral blood (2 cc) was obtained by aseptic precautions in heparinised syringe from 10 healthy donors of age group 25 35 years. Three culture tubes coded as "1", "2", and "3" were filled with 5 ml MEM media supplemented with human cord blood serum, antibiotics, glutamine and phytahaemagglutinin (PHA- L, mitotic stimulant), 0.5 ml blood was added to each culture tubes. Cultures were incubated at 37o C for period of 68 hrs, 70 hrs and 71.5 hrs for "1", "2", and "3", respectively

Experiments were carried out specific concentration and exposure timing of colchicine to obtain chromosomes at metaphase stage (Table 1).

Cultures were then processed according to standard procedure and slides prepared were coded and analyzed for chromosomal length in different conditions that were given. The length of longest chromosome was used as parameter to see the effect of various exposure timing of colchicine.

About 40 50 metaphase spreads were

screened at random for each sample. Finally 30 good spreads were selected for measuring length of longest chromosome. Length of longest chromosome was measured with ocular micrometer scale under oil immersion lens.

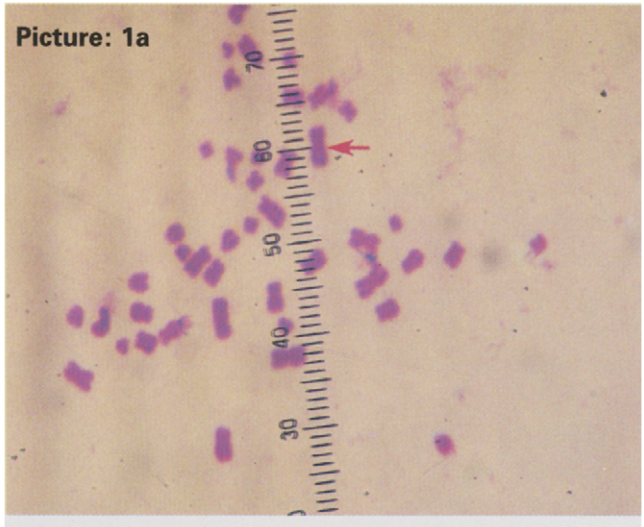
Effect of colchicine exposure on short chromosomes was not observed in the study.

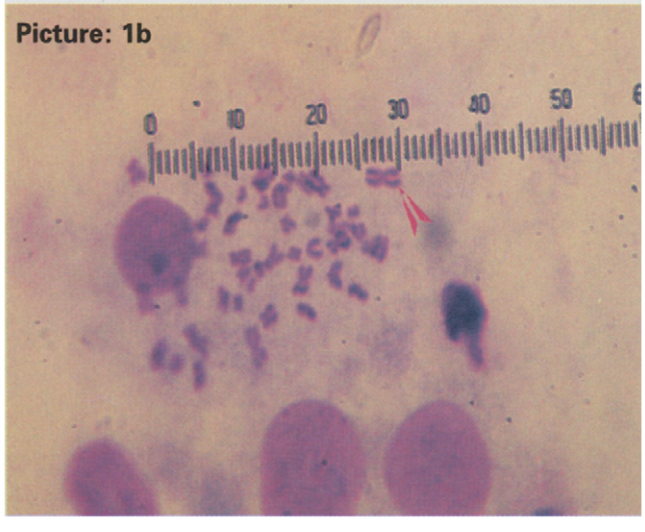
OBSERVATIONS AND RESULTS

Experiments with same concentration of colchicine (10 μg /ml) and different exposure time gave results as shown in Table-2.

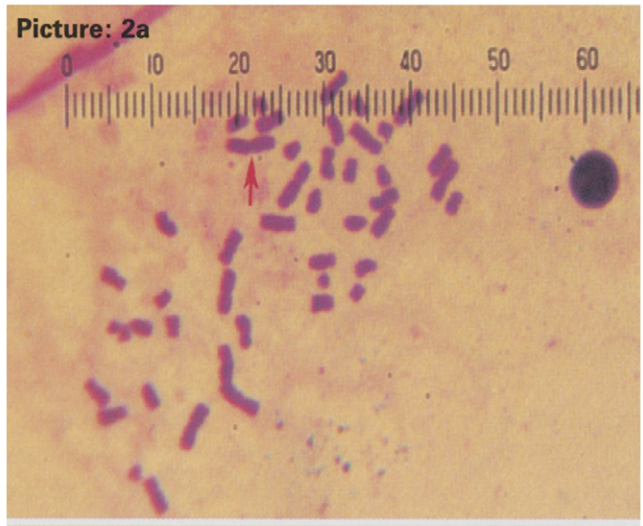
Picture 1a and 1b show the length of longest chromosome from a metaphase spread of sample exposed to colchicine for 4 hours with mean length ranging from 3.80-4.90 μm.

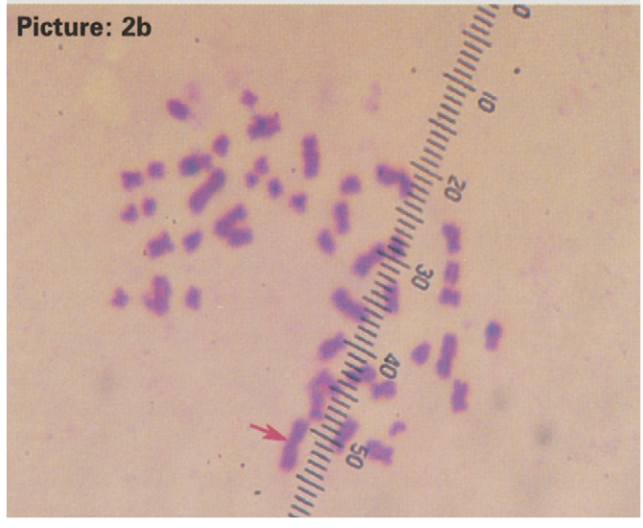
Picture 2a and 2b show the length of longest



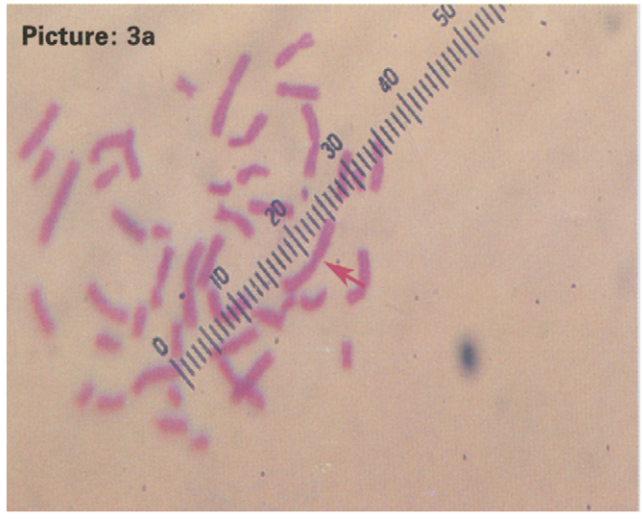


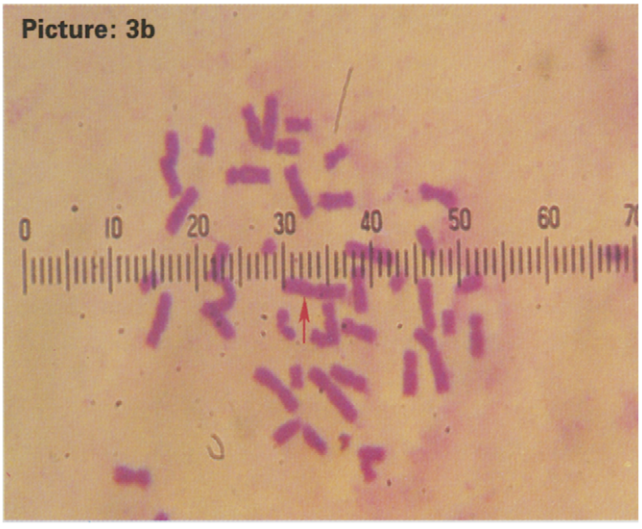
Photograph showing length of longest chromosome from metaphase spread exposed to colchicine for 4 hours





Photograph showing length of longest chromosome from metaphase spread exposed to colchicine for 2 hours





Photograph showing length of longest chromosome from metaphase spread exposed to colchicine for1/2hour

Culture tube no.	Addition of colchicine at ----hr of culture	Total exposure period for colchicine	Incubation of culture in incubator
1	68th hour	4 hour	72 hour
2	70th hour	2 hour	72 hour
3	71st hour 30 mints	½ hour	72 hour

Table-1
Time of addition and total exposure period of colchicine to lymphocyte culture

No.	Culture tube –1 (in µm)	Culture tube –2 (in µm)	Culture tube-3 (in µm)
1	4.53	5.50	6.03
2	3.50	5.10	7.46
3	3.90	5.00	6.76
4	4.20	5.10	6.86
5	4.70	5.00	6.46
6	4.01	6.00	7.70
7	4.13	5.00	7.10
8	3.90	5.00	6.80
9	3.80	5.00	5.53
10	4.90	5.20	5.50

Table 2
Mean length of longest chromosome from each group of culture

Culture tube no.	Mean length range (in µm)
1	3.80 – 4.90
2	5.00 – 6.00
3	5.53 – 7.70

Table 3
Mean length of longest chromosome from each culture

	Difference between length of chromosomes of culture 1 &2	Difference between length of chromosomes of culture 2 &3	Difference between length of chromosomes of culture 1 &3
Mean difference	1.0130	1.4330	2.4460
S.D.	0.5292	0.8191	1.0790
S.E.	0.1674	0.2590	0.3412
't' value	6.0500	5.5320	7.1680
'P'value	P<0.001	P<0.001	P<0.001

Table-4
Statistical data showing comparison of length of chromosomes of all the culture

chromosome from a metaphase spread of sample exposed to colchicine for 2 hours with mean length ranging from 5.00-6.00 µm.

Picture 3a and 3b show the length of longest chromosome from a metaphase spread of sample exposed to colchicine for 1/2 hours with mean length ranging from 5.53-7.70 µm

Table 3 shows the range of mean length of longest chromosomes from each culture group. The mean length of longest chromosomes from each group of culture tube was compared with each other Table-4 shows the data of comparison among all the cell culture groups. Mean length and S.D. were worked out. 't' test were applied ,to see difference among length of longest chromosomes in cells from culture tube-1,2 and 3 't' test yielded significant results among all the three comparison. Statistical results of present study shows that shorter exposure period of colchicine gives longer chromosomes and longer exposure period gives condensed chromosomes both of which can be used for further cytogenetic study.

DISCUSSION

In present study we observed the chromosomes in more condensed state when colchicine was added at 68th hour of incubation in culture -1. In culture -3 colchicine was added ½ hour before completion of incubation period of 72 hour.

Length of longest chromosomes was found longer in cells of culture -3 in compare to that of other cell culture group.

Chin Yuet Meng[6] in his cytogenetic study of CML he stated that a longer exposure of colchicine results in more metaphase spread but in these spreads the chromosomes will be shorter because the chromosomes condense as they progress through metaphase. In present study also longer exposure period of colchicine gave condensed chromosomes and shorter duration of exposure gave longer chromosomes.

V.Nandini &S.K.Shyama[7] showed with their experiments that while using various concentrations and various exposure timing of colchicine to lymphocyte culture gave best results at final concentration of 10microgram/ml and an exposure period of 15 minutes.

De laat found a direct correlation between chromosome condensation and prefixing temperatures. Low temperature treatment of mitotically dividing cells resulted in contracted & sticky chromosomes

Condensation of chromosomes depends upon concentration of metabolites in different phases of cell cycle[8]; phases of cell cycle itself; prefixed temperature and humidity.

Longer the length of chromosomes will give good results in GTG banding and there by good results in karyotyping also.

CONCLUSION

Statistical results of present study shows that longer period of exposure of colchicine yield condensed chromosomes in comparison to shorter exposure period which yields longer chromosomes.

Longer chromosomes in comparison to shorter ones give better results for karyotyping and banding techniques.

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