



Original Research Article

Study on the Effect of Different Hypotonic Solutions on Chromosomes during Mitosis

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ABSTRACT

The precise role of hypotonic solution is that it causes the nuclei to swell and the cell bursts and the chromosomes are opened. In routine karyotyping procedure, cells are exposed to Ohnuki solution apart from KCl as hypotonic solutions. The duration of exposure also varies from 8 to 10 minutes. To get better quality of healthy chromosomes different hypotonic solutions have been studied. It was observed that 0.33 % of NaCl incubated at 37°C has been found to be better than the KCl and Ohnuki solutions in getting the healthy chromosomes.

Keywords: Chromosomes, Hypotonic solution, karyotyping.

INTRODUCTION

From the cytogenetic point of view it has been observed that a hypotonic solution has lower concentration of solutes than its surroundings, so in an attempt to balance the concentration, water will rush into the cell causing swelling.

Tonicity is a measure of osmotic pressure gradient of two solutions separated by a semi permeable membrane. It is commonly used when describing response of cells immersed in an external solution. Like osmotic pressure, tonicity is influenced only by solutes. ^[1]

Here in karyotyping we use hypotonic solution for the rupture of cells. The extra cellular solutes are less concentrated than intracellular solutes. So the water rushes inside the cells to reach the

equilibrium causing the swelling thereby bursting out of the cells releasing the chromosomes.

The time of exposure to hypotonic treatment is critical as the concentration, temperature and type of hypotonic solution employed varies. Over treatment, leads to over spreading with rupture of cell membrane thus leading to random loss of chromosomes. Under treatment leads to inadequate spreading and inability to distinguish individual chromosomes because of overlaps. ^[2]

Lymphocyte cultures are routinely prepared by employing the modified method of Arakaki & Sparkes. ^[3] This study was aimed to see the effect of various hypotonic solutions on lymphocyte culture during mitosis.

MATERIALS & METHODS

Peripheral blood was obtained by aseptic precautions in heparinised syringe from 10 healthy donors of age group 20 to 40 years.

Three culture tubes coded as “1”, “2” and 3 were filled with 5 ml RPMI media (GIBCO) with NBC serum, antibiotic and PHA (supplied by shreyas solutions - Bangalore). 0.5 ml of blood was added to each culture tubes. Cultures were incubated at 37°C for a period of 72 hours. Experiments were carried out by using 0.33% NaCl, 0.56% KCl and ohnuki solution as hypotonic solutions for “1”, “2” and “3” respectively.

Cultures were then processed according to the standard procedures and slides prepared were used and analyzed for good spreads in different hypotonic solutions that are given.

About 20 to 30 good spreads with healthy chromosomes were screened at random for each sample. Finally 25 good spreads were selected.

OBSERVATIONS & RESULTS

Experiments with different hypotonic solutions such as 0.33% NaCl, 0.56% KCl and ohnuki gave results as shown in the Table 1.

Tube No	Hypotonic Solution	Concentration	PH	Time (in Minutes)	Observation of Healthy Chromosomes
1	NaCl	0.33 %	7	8	Very Good - + + +
2	KCl	0.56 %	7	8	Good - ++
3	ohnuki	0.83 %	6.8	10	Very Good - + + +



Figure (1) shows better quality of healthy chromosomes by using 0.33% NaCl

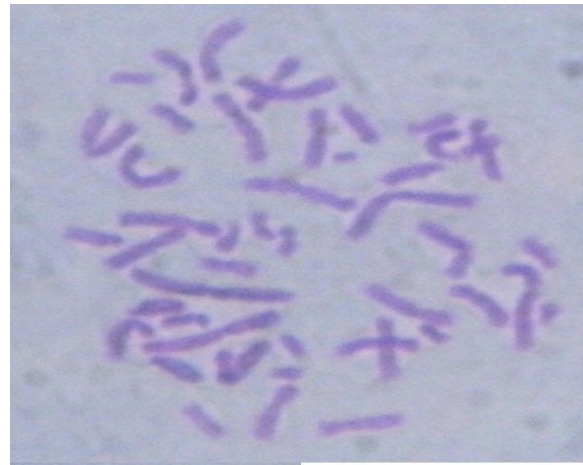


Figure (2) shows fair quality of chromosomes by using 0.56% of KCl



Figure:3 shows good quality of healthy chromosomes by using ohnuki solution. The result of the present study shows that better quality of healthy chromosomes can be obtained by using 0.33% NaCl which can be used for further cytogenetic study.

DISCUSSION

In the present study we observed that chromosomes are good in quality when 0.33% NaCl was added during harvesting in culture. ⁽¹⁾ In culture ⁽²⁾ KCl was added which gave a fairly good quality of chromosomes compared to culture. ⁽¹⁾

Human mitotic metaphase plates are prepared by using KCl as hypotonic solution by Moorhead et al ⁽⁴⁾ Chauffaile Mde .L et al developed a study of simplified method for the analysis of cellular karyotype & phenotype. They used hypotonic solution

formed from glycerol, NaCl, KCl, CaCl₂, MgCl₂ and sucrose. ⁽⁵⁾

Better quality of healthy chromosome will give good results in karyotyping.

CONCLUSION

The present study shows that the 0.33% NaCl can be used as a hypotonic solution to get better spreads of healthy chromosomes in comparison with ohnuki KCl solutions.

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