

The ideal time to add Colcemid to obtain a division of the lymphocyte cells

Minnatullah Abbas Al -Ammar¹; Sada Jasim Abdulameer²

College of Education for Pure Sciences, University of Wasit, Iraq

minnatabbas1101@uowasit.edu.iq¹, sabdulameer@uowasit.edu.iq²

Abstract: This research was conducted to determine the optimal conditions for the addition, and withdrawal times of colcemid by culturing colcemid in different media and to examine the effect of colcemid on lymphocyte proliferation and chromosome quality. Peripheral blood (PBL) was collected in a short-term culture. The colcemid incubation period is one factor that may affect experimental results. Routine karyotyping procedures for lymphocyte cultures (blood cultures) include exposing cells to colchicine to stop metaphase cell division and visualizing chromosomes. The exposure time to colchicine in previous research is 1 to 2 hours. After adjusting the duration of exposure to colchicine to improve chromosome quality. Although genetic/laboratory experiments were standardized by division, different durations of colchicine exposure were performed to obtain longer chromosomes with split chromosomes. We observed that shorter periods of colchicine exposure resulted in better-quality chromosomes, while longer periods of colchicine exposure resulted in fertilized chromosomes.

Keywords: colcemid effect, lymphocyte proliferation, chromosome quality

introduction

Colcemid (Demecolcine), a colchicine derivative, is a potent inhibitor of mitosis. Colcemid binds to tubulin protein and metaphase arrest cells for karyotyping assays. Colcemid induces apoptosis and can be used for cancer research. It separates chromosomes for cytogenetic investigations and interrupts the cell cycle and growth because it prevents spindle formation during mitosis. Colcemid solution is a medical compound. Anti-mitotic properties of colchicine and tubulin by tubulin binding, microtubule assembly of colchicine block, and polymerization, which is the best-researched therapeutic modality (Arigony, & Henriques.2013), (Vladimír, *et.al.*2020). From the cytoskeleton. Microtubules act in a variety of cellular functions, Including shape maintenance, intracellular trafficking, cytokine and chemokine secretion, cell migration, ion channel control, and cell division. Colcemid, a traditional antibacterial drug, prevents cells from entering anaphase. Tubulin-Colcemid complex forms are less reversible with tubulin soluble, which binds to the ends of microtubules and prevents elongation of the micro polymer. Colcemid inhibits microtubule development at low doses and promotes the depolymerization of microtubules at higher doses (Higdon 2016). The inflammatory actions of colchicine are associated with the breakdown of microtubules and the cellular activity of leukocytes.

At the cellular level, Colchicine is an antiapoptotic agent that inhibits mitosis by blocking the polymerization of tubulin, a protein of constituent microtubules, thus perturbing those processes that depend on microtubule function such as intracellular cell movement, cell polarity, and mitosis. Colchicine is a classic anti-mitotic drug that inhibits mitotic cells in prophase. The mechanism is that it binds to soluble tubulin to form tubulin-colchicine complexes in a poorly reversible manner, which then bind to the ends of microtubules to prevent elongation of the microtubule polymer/inhibit microtubule aggregation, thus interfering with mitotic spindle formation and arrest. Cell division as well as inhibition of cell migration (Kidani 2013). The most common adverse effect of colchicine is diarrhea, which affects rapidly proliferating intestinal epithelial cells. Colchicine inhibits microtubule aggregation and thus disrupts inflammatory particle activation, microtubule-based inflammatory cell chemotaxis, leukocyte and cell generation, and phagocytosis. Many of these cellular processes can be found in other diseases that involve chronic inflammation. Cold and colchicine's most striking effect is stopping mitosis when the chromosomes reach their terminal anaphase. This arrest leads to an accumulation of cells at this stage (Kratz, *et.al.*2014). One of the factors that may affect the results of the trial is the colcemid incubation period. Therefore, the research aims to determine the appropriate time in the use of colchicine for any special medium for the culture of lymphocytes, so that it is the ideal time to obtain a large number of cell divisions and the shape of clear chromosomes.

Materials and method

The study began on August 1, 2022, until January 1, 2023, in the laboratories of the College of Education for Pure Sciences, Department of Life Sciences, Graduate Laboratory. The research included the study of the transplantation of lymphocytes from whole blood of a blood sample taken from a healthy person according to clinical chemistry analyses. Different methods of culturing lymphocytes were tested systematically, then different times of adding colchicine were tested to obtain the best cell division and the nature of their chromosomes, and compared to the methods, starting with the general method and then with the methods available on commercial kits these four methods as according to Table (1), the time of adding colchicine was different periods, natural media were compared with commercial media available in the local market (RPMI1640, LymphoPrime, Complete Medium for Peripheral Blood Lymphocytes Cat. No. LPR-B (100 ml), Chromosome Kit and Medium P, and CHANG Medium MF). Then the first, traditional method was used in lymphocyte culture, according to the method (Lefort C.T.and, Kim M. (2010). The modified method-5 adds only a quarter of an hour before the end of the incubation period, 72 hours.

Table (1) the differences in Timing colcemid between methods 1,2,3and 4.

steps	Method 1	Method 2	Method 3	Method 4

Timing of colcemid exposure	Colcemid treatment Half an hour before the end of planting time for 72	colcemid solution (10 µg/ml) for 1 to 2 hours	Colcemid (10 µg/ml) and incubate at 37°C for 90 minutes	Colcemid (10 µg/mL) to Incubate tubes at 37°C, for 45 minutes.
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Results

Effects of the cultivation media on the peripheral blood lymphocytes based on the blast cell, mitotic, and metaphysis indices using method-5 (differences of Timing colcemid). The present findings demonstrated significant ($p<0.0001$) differences in each blast, mitotic, and metaphase indices between the cultivation media utilized in method-5. Under this situation, the CMF, CCLP, and EOLCP, revealed the highest significant ($p<0.0001$) increases in the blast cells (31.991, 31.230, and 31.140), respectively. In addition, the blast index in the MAL (27.250) and R1640S (27.245) revealed the 2nd significant ($p<0.0001$) highest among the nutritional media. However, the remaining media showed comparable reads to indicate the lowest significant ($p<0.0001$) increases in this parameter.

For the mitotic index, the CCLP, EOLCP, and CMF demonstrated the highest significant ($p<0.0001$) elevations in the blast cells (3.355, 3.231, and 3.145), respectively. In addition, the blast index (2.911) in the MAL was declared the 2nd significant ($p<0.0001$) highest among the nutritional media. However, the remaining media indicated comparable records to indicate this parameter's lowest significant ($p<0.0001$) scores. In the case of metaphase, the CCLP, CMF, and EOLCP unveiled the highest significant ($p<0.0001$) increases in the blast cells (9.525, 9.450, and 9.440), respectively. In addition, the blast index (2.381) in the albumin was declared the 2nd significant ($p<0.0001$) highest among the nutritional media. However, the remaining media recognized comparable reads to indicate this parameter's lowest significant ($p<0.0001$) increases as in Table (2,3,4,5, and 6) and Fig. (1).

Table (2): Cytogenetic analysis of peripheral blood lymphocytes according to method-1 using different media.

Cytogenetic parameters	Synthetic medium (RPMI 1640)	Complete medium	Natural medium	P-value
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	With serum	Free serum	CAPRICORN Medium Lymph-prime	Euro O Lone Chromosome Medium P	CHANG Medium MF	Albumin	lipid	mix	
Blast index	26.200	25.330A	30.245B	31.100	30.250B	25.110C	22.250	25.250A, C	<0.0001
Mitotic index	1.240	1.190	3.264D	3.266D	3.255	1.169	1.150	1.210	<0.0001
% of cells in metaphase	1.110	1.930	6.340	7.890	7.682	2.091	1.891	1.123	<0.0001
Monitor healthy chromosomes (karyotype)	Healthy Chromosomes								

Similar letters: no significant ($p>0.05$) difference (horizontal comparisons).

Different letters or no letters: significant ($p<0.0001$) difference (horizontal comparisons).

Table 3: Cytogenetic analysis of peripheral blood lymphocytes according to method-2 using different media.

Cytogenetic parameters	Synthetic medium (RPMI 1640)	Complete medium	Natural medium	
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	With serum	Free serum	CAPRICORN Medium Lymph-prime	Euro O Lone Chromosome Medium P	CHANG Medium MF	Albumin	Lipid	Mix	P-Value
Blast index	26.99	25.431	31.452	30.443	30.236	26.210	23.532	25.451	<0.0001
Mitotic index	2.322	1.532	3.536	3.451	3.561	2.980	1.871	2.169	<0.0001
% Of cells in metaphase	2.120	1.856	7.540	6.650	7.400	2.210	1.901	1.951	<0.0001
monitor healthy chromosomes (karyotype)	Healthy Chromosomes								

No letters: significant ($p < 0.0001$) difference (horizontal comparisons)

Table 4: Cytogenetic analysis of peripheral blood lymphocytes according to method-3 using different media.

Cytogenetic parameters	Synthetic medium (RPMI 1640)		Complete medium			Natural medium			P-Value
	With serum	Free serum	CAPRICORN Medium Lymph-prime	Euro O Lone Chromosome Medium P	CHANG Medium MF	Albumin	Lipid	Mix	
Blast index	26.540	25.780	31.561	31.760A	31.671A	26.320	23.450	25.00	<0.0001
Mitotic index	2.230	1.670	3.661	3.789	3.540	1.811	1.900	2.081	<0.0001
% Of cells in metaphase	2.90	1.822	7.019	8.010	7.900	1.930	1.890	2.110	<0.0001
monitor healthy chromosomes (karyotype)	Healthy Chromosomes								

Similar letters: no significant ($p > 0.05$) difference (horizontal comparisons).

Different letters or no letters: significant ($p < 0.0001$) difference (horizontal comparisons).

Table: 5: Cytogenetic analysis of peripheral blood lymphocytes according to method-4 for different media.

Cytogenetic parameters	Synthetic medium (RPMI 1640)		Complete medium			Natural medium			P-Value
	With serum	Free serum	CAPRICORN Medium Lymph-prime	Euro O Lone Chromosome Medium P	CHANG Medium MF	Albumin	Lipid	Mix	
Blast index	26.012A	25.540	31.190	31.890	32.190	26.901	23.320	26.010A	<0.0001
Mitotic index	2.380	1.901	3.289	3.890	3.786	2.030	1.781	2.981	<0.0001
% Of cells in metaphase	2.760	1.980B	8.320	7.880	8.540	1.880	1.980B	2.112	<0.0001
monitor healthy chromosomes (karyotype)	Healthy Chromosomes								

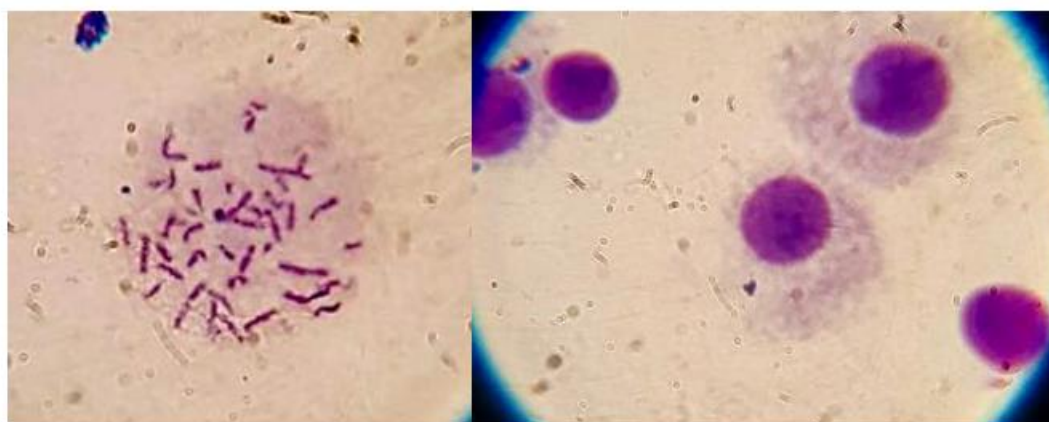
Similar letters: no significant ($p>0.05$) difference (horizontal comparisons).

Different letters or no letters: significant ($p<0.0001$) difference (horizontal comparisons).

Table (6) Cytogenetic analysis of peripheral blood lymphocytes according to method-5 for different media.

Cytogenetic parameters	Synthetic medium (RPMI 1640)		Complete medium			Natural medium			P-Value
	With serum	Free serum	CAPRICORN Medium Lymph-prime	Euro O Lone Chromosome Medium P	CHANG Medium MF	Albumin	Lipid	Mix	
Blast index	27.245	26.332	31.230	31.140	31.991	26.800	23.190	27.250	<0.0001
Mitotic index	2.312	2.450	3.355	3.231	3.145	2.450	2.021	2.911	<0.0001

% Of cells in metaphase	3.210	2.198	9.525	9.440	9.450	2.381	2.250	2.325	<0.0001
monitor healthy chromosomes (karyotype)	Healthy Chromosomes and good								



(A)

(B)

Fig (1): (A) shows lymphocyte chromosomes at the mitotic stage.

(B) shows Five swollen cells ready to divide

Discussion

It has been observed that by shortening the period of exposure to colchicine, better quality of chromosomes can be obtained while a longer period of exposure to colchicine gives condensed chromosomes (Koyani,&, Saiyad 2013) and this is consistent with the results of this research. While in other research, the result was the mitotic period and prevented the cells from entering the equatorial phase. Colcemid treatment at 0.02 to 0.04 $\mu\text{g/mL}$ for 2 hours was sufficient to elicit an asynchronous growth of synchronized cells. Mitotic clusters increased by approximately 40% during this treatment compared to the untreated control culture. The purity of the harvested cells was over 85%. The average generation time did not differ significantly between the reversible and untreated cells. However, treatment with colcemid delayed the DNA increase by about 2 hours during the synchronized growth (Preeti, Koyani, Saiyad. 2011) (Lefort C.T., Kim M. (2010). In another research, which agreed with the current research, it was found that a routine karyotyping procedure of lymphocyte culture (blood culture), and exposure of cells to colchicine arrested cell division in metaphase to visualize chromosomes. The duration of exposure to colchicine routinely varies from one to two hours. Both concentration and exposure period can be changed for a better quality of chromosomes. During the standardization of the genetic section/research

laboratory, different periods of exposure time to colchicine were made to get longer chromosomes with dividing chromosomes. It was observed that by shortening the exposure period to colchicine longer and better can Chromosomal quality is obtained while a longer period of exposure to colchicine gives a chromosomal condenser (Preeti. *et.al.*2011).

Conclusion Through the results of the research, it was found that the use of colcemid to stop cell division depends on the time of addition and the duration of addition to the different culture media used, and the research concluded that only 15 minutes is the best time period to obtain the highest rate of division and the best shape of the chromosomes.

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