

# Comparison and Frequency of Cell Cultured in 48-h and 72-h Mitotically Treated with Phytohemagglutinin-M: Both Promise for Normal and Cancer Cells

## Abstract

The aim of the present study is hence to comprise of mitotic index outcome in the individuals of normal volunteers involved in two different period of culture with effect of Phytohemagglutinin-M. Comparison of healthy human donors lymphocytes were cultured for 48-h and 72-h and using conventional karyotype technique with minor modification. Mitotic index and cell proliferation frequency were analyzed in the individuals of normal blood donors after preparing of colchicine to cultured metaphases 2 h before cell fixation. The metaphase indices at 48-h and 72-h culture period resulted a perfect yield product. Lymphocytes populations in 72-h laboratory work were more than 48-h cultures (2.73% at 72 h vs. 1.48% at 48 h,  $P \leq 0.05$ ). The present research indicates significant comparisons for laboratories and individuals to be set up. With effect of these results, it may help in time-consuming and short time period for patient, as well as fresh suggestion for health-care management decision. Reagent, manual, budget, time for diagnosis, and health-care fulfillment may resolve of this proposal.

**Keywords:** 48-h and 72-h cultured lymphocyte, cell proliferation index, comparison, normal donor, phytohemagglutinin

## Introduction

Cytogenetics is the science of arranging and preparing all the metaphases of an chromosome populations.<sup>[1]</sup> Chromosome preparation is more essential for staining that results toward features of structural and numerical for all metaphases.<sup>[2]</sup> Cytogenetic are currently is the panel of useful diagnostic for genetic disorders, specific birth defects, and even leukemias and cancers.<sup>[3,4]</sup> Cytogenetic are arranging from metaphase cells that has been arrested in the prometaphase and metaphase section of the cycle cell, where metaphases seems their highest condense compare to prometaphase performance.<sup>[5,6]</sup> The number of specimen shall be utilized as a source of these metaphases. Genetics are prepare from whole blood materials,<sup>[7]</sup> biopsy of skin,<sup>[8]</sup> diagnosis of prenatal,<sup>[9]</sup> chorionic villus or amniotic fluid specimens<sup>[10]</sup> are manipulate as the specimen of cells.

Normal karyotype among patients with acute myeloid leukemias is rare, accounting

for <20% of cases reported in the current literature.<sup>[11]</sup>

The presence of karyotypic abnormalities, with aneuploidies that are currently found in several different cancers and others syndromes are specific and nonrandom to individual tumors and tissues/organs of origin.<sup>[12]</sup>

Instead, the fact that the tissue/site of origin is important in determining karyotypic patterns in cancer cells indicates that selection of specific karyotypes must also depend on the specific biology and physiology of cells from different tissues/organs. Indeed, microarray analysis of cells from different tissues shows tissue-specific gene expression patterns in normal diploid cells.<sup>[13]</sup> Most cancer cells are aneuploid and display a chromosomal instability phenotype; chromosomal instability can be caused by numerous mechanisms.<sup>[14]</sup>

The exact timing for mitotic cycle is well established; it can be explain that any different timing culture in the growing rate of the cell culture are because of differences

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in the chromosome indices and not due to manipulation in the exact time of mitosis.

Aberration in cell cycle mitotic index are established to be upon the culture of both situation and the respond of the lymphocytes of each person to ability of phytohemagglutinin (PHA) effects in times period differences.<sup>[15,16]</sup> The index mitosis and kinetic of cell cycle without lymphocytes are exhibited to differ herald among donors of blood specimen.<sup>[16]</sup> With effect of these results, it may help in time-consuming and short time period for patient, as well as fresh suggestion for health-care management policies.<sup>[17,18]</sup> Reagent, manual, budget, time for diagnosis, and health-care fulfillment may resolve these investigations.<sup>[19,20]</sup> The present research work represents a detailed study of mitogen phytohemagglutinin (PHA-M) properties of cell populations involved in two different periods of culture.

## Materials and Methods

### Patients and sample collection

The present research study with 100 adult normal volunteers of either sex at Shahid Beheshti University of Medical Science was elaborated between 2015 and 2018. These healthy subjects were chosen from a common blood donor who had not infected and exposed to chemical materials, different rays, reagent substances, drugs, or other that could hazard mitotic alteration percentage.

In one sample, 0.5 ml to 1.0 ml peripheral blood was taken and manipulating cell culture procedure as; (a) 48-h stimulated culture test and (b) 72-h were used stimulation culture technique. The cultures were set up here after a modified method. For present cell culture procedure, 3–5 × 10<sup>6</sup> cells was cultured in 4 ml tissue culture media (Gibco-BRL Grand Island, RPMI 1640, NY, USA) added with 10%–14% inactivated with heat of fetal calf serum (Gibco-BRL Grand Island, RPMI 1640, NY, USA) at 37°C in 5% CO<sub>2</sub> condition. The standard of metaphase cell growing from 48-h stimulations as well as from 72-h cultures was noted based on the references with minor modified method. Finally, the cultured lymphocytes were treated with colchicine (Grand Island, NY, USA, Gibco-BRL), total volume 10 µg/ml, and then incubated at 37°C for 3 min. The volume of the culture flask was then centrifuged for 8 min at 1000 rpm in 10 ml of 75

KCl mM (0.55%) resuspended and prewarmed to 37°C for 20 min. In this step, 1 ml of standard fixative (1:3 acetic acid: methanol) was increased into the flask, and the step of fixation was done 4 times. Twenty slides were arranged for every sample test and stained for 3 min with Giemsa stain. Examination of slides was tested with a Nikon light microscope. Hundred spread of metaphases was evaluated for cytogenetic study. Metaphases were defined based on the ISCN.<sup>[21]</sup>

### Mitotic index determination

The mitotic indices of cultures harvested after 48 and 72 h of incubation were calculated as the number of metaphases among 1000 randomly scanned lymphocytes and expressed in percentages. In other words, mitotic indices were compiled from counts of 1000 mononuclear cells.<sup>[22]</sup>

### Statistical analysis

The protocol for statistical package for research work v. 21.0 for Windows (Chicago, IL, USA) was used for the statistical analysis. Differences in mitotic index frequencies between the two periods culture comparison were used Chi-square and Fisher's exact tests.

## Results

### Data of mitotic observation

Observation of present investigation of mitotic activities and chromosome index in lymphocytes of donors peripheral blood have been resulted the 48-h and 72-h effect of similar culture concentrations.

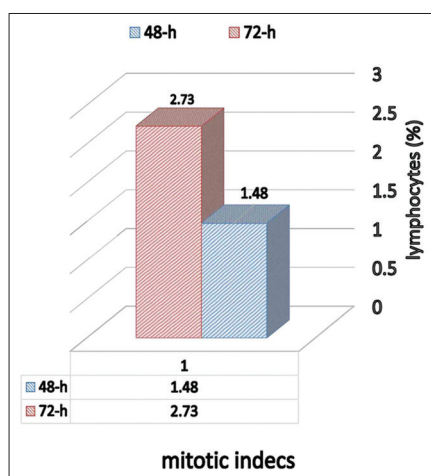
A brief study of the chromosome indices and karyotype data is exhibited in Table 1. As resulted, the chromosome index of the harvested cultures at 72 h was significantly higher than the 48 h specimen (2.73% at 72 h vs. 1.48% at 48 h,  $P \leq 0.05$ ). The different karyotyping yields between the normal female and male blood donors were almost similar in the current investigation at the both culture times. The vast variety in cell population index of harvested lymphocytes was evaluated among blood volunteers.

The culture conditions were equal for both harvesting times. The comparison of mitotic of each volunteer with effect of PHA stimulation and the mitotic index production, the mitotic populations were noticed in a different culture

**Table 1: Mitotic indices of cultured for 48-h and 72-h, Mitotic indices were based on 1000 cells per subjects and expressed as percentage**

	48 h (%) Age (years)		72 h (%) Age (years)	
Number of subjects	100		100	
Mitotic index	1.48		2.73 <sup>a,*</sup>	
Male blood donor	56 (56)	43.8±5.7	56 (56)	43.8±5.7
Female blood donor	44 (44)	38.7±4.9	44 (44)	38.7±4.9
Total subject	100		100	

<sup>a,\*</sup>Significantly different from 48 h frequency \* $P \leq 0.05$



**Figure 1: Comparison of frequency of normal human lymphocytes cell cultured in 48-h and 72-h**

frequency. Furthermore, no qualitative difference was set up between the two types of cultures, observed in both culture periods. Overall, in the present study a higher products in metaphase index of all blood donor sample was investigated. However, many mitotic products with higher degree of metaphases contraction were noticed. A nonrandom selection of well-prepared slides including cell populations index were chosen in these culture conditions. An increase in the population of mitotic with abnormal metaphases such as gap and break were also observed due to unexpected reason Figure 1.

## Discussion

It was considered that the 48-h cell cultures consist of division first mitosis cell cycle. In other hand, as many as of geneticist has used 72-h as the ideal cell culture period for routine diagnosis.

Most researchers in the laboratories investigate karyotypes, prepared from mitotic cells that have been treated PHA stimulus for 72-h in the prometaphase or metaphase stage of the cycle cell, when chromosome consider their most condensed performances.<sup>[5,8,10]</sup> It would be prudent to take advantage of higher metaphase indices of cultures and treated with PHA at 72-h.<sup>[22]</sup> In accordance with reports in the current literature and the result of this project performed on all healthy blood donors as described earlier,

the metaphase index of the PHA-treated cultures and harvested at 72 h was significantly higher than the 48-h samples (2.73% at 72 h vs. 1.48% at 48 h,  $P \leq 0.05$ ). On the other hand, the metaphase index and kinetic cell cycle of untreated with PHA, metaphases production were noticed with different results for normal samples from blood donor was reported in the current literature.<sup>[23,24]</sup> The lymphocytes subpopulation cellular is of good idea from a standard standpoint till it is the section of the population of lymphocytes when it contributes to develop in the lymphocytes number. Therefore, any differences in the cell growing percentage of the culture are resulted to be mirrored by the presence of lymphocytes undergoing cellular divisions that could be reasonably approximated by the metaphases population of cell growth.<sup>[11]</sup> In spite of the cell cycle kinetic findings, lymphocyte growth activity were seen in the cultures flask. Incubates for <48 h cannot be possible owing to the less of an average count of mitotic indexes.<sup>[25,26]</sup> Evaluation of the percentage of chromosome structural abnormalities (CA) in different culture periods mostly affected by radiation for circular metaphases is now currently manipulated in karyotyping evaluated on human individuals.<sup>[18]</sup> According to the results reported by some workers, it is reasonable to express that 48 h cannot be the difficult period in the detection of spontaneously happening chromosomal abnormalities in healthy normal volunteers. Despite the possibility that as many as abnormal metaphases may be eliminated or many abnormalities might be restituted from 48-h to 72-h gap period time, the results remind that a 72 h culture does not influence significantly the spontaneous abnormal karyotype results in this field. Perhaps, the cell proliferating populations at the 48-and 72-h culture period maintain a significant fix result parameter. As there is not much differences in abnormal results, it could be prudent to make advantages of much more mitosis indices of harvested at 72 h.<sup>[22]</sup> In other words, as many as the chromatid and other abnormal metaphases would be high if the lymphocytes growth times with 2 or more cell cycles growth production.<sup>[27,28]</sup> In spite of the lymphocyte kinetic findings. Karyotyping research performed on harvest incubated for <48-h cannot be possible owing to the deficit of an available number of chromosome indexes.<sup>[22,29]</sup>

In conclusion, these results exhibit correct comparisons among individuals and laboratories to be set up. With effect of these results, it may help in time-consuming and short time period for patient, as well as fresh suggestion for health-care management decision. Reagent, manual, budget, time for diagnosis, and health-care fulfillment may resolve this proposal.

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Nil.

### Conflicts of interest

There are no conflicts of interest.

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