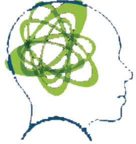

Cell, Gene and Therapy

Research Article

Cell Cultured Procedure in Normal Human Lymphocytes for Stem Cell Therapy

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Background: Abnormalities in mitosis parameter and cycle cell program are announced to be based on both the cell proliferation conditions and the condition of the metaphases of each person to respond to Phytohaemagglutinin (PHA) working condition. The aim of the current research is hence to comparison of mitotic index outcome in the individuals of normal volunteers involved in two different period of culture with effect of PHA.

Methods: Comparison of healthy human donors lymphocytes were cultured for 48-h and 72-h and using conventional karyotype technique with minor modification.

Results: Mitotic index and cell proliferation frequency were analyzed in the individuals of normal blood donors after preparing of colchcine to cultured metaphases 2 hours before cell fixation at. The metaphases indics at 48-h and 72-h culture period maintained a perfect yield level. Lymphocytes populations in 72-houres laboratory work were more than 48-h cultures (2.73% at 72 hours vs. 1.48% at 48 hours, $p \leq 0.05$).

Conclusion: 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. **Cell, Gene and Therapy, Vol.1, Number 2, summer 2020; 73-79**

Keywords: Comparison; Cell proliferation index; 48-h and 72-h cultured lymphocyte; Phytohaemagglutinin (PHA); Normal donor

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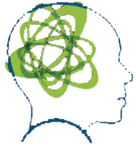

Introduction

Cytogenetics is the science of arranging and preparing all the metaphases of a population[1]. Chromosome preparation are more common staining manipulation that results towards features of structural for all metaphases[2]. Cytogenetic are currently is the panel of information diagnostic for genetic disorders, specific birth defects, and even leukemias and cancers[3,4]. Cytogenetic are arranging from metaphase cells that has been stopped in the prometaphase and metaphase part of the cycle cell, where metaphases seems their highest condense performance[5,6]. The number of of specimen shall be utilized as a source of these metaphases. Cytogenetics are always initiated from whole blood materials[7], biopsy of skin[8], diagnosis of prenatal[9], chorionic villus or amniotic fluid specimens[10] are preparing as the specimen of cells.

The exact timing for mitotic cycle is well established, it can be inferred that any different timing in the all aspect growing rate of the cell culture are because of differences in the chromosome indics and not to change manipulation in the exact time of mitosis.

Aberration in cell cycle kinetics and mitotic index are established to be upon the culture of both situation and the respond of the lymphocytes of each individual to ability to Phytohaemagglutinin effects in times period differences[1,12]. The index mitosis and kinetic of cell cycle without lymphocytes are exhibited to differ herald among donors of blood specimen[12]. 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[13,14]. Reagent, manual, budget, Time for diagnosis, and health care fulfillment may resolve of this investigations [15-16]. The present research work will represents a detailed study of mitogen Phytohemagglutinin (PHA-M) properties of cell populations involved in two different period of culture.

Method

The present research study with 100 adult normal volunteers either sex in the department of cell biology and Anatomy at Shahid Beheshti University of Medical Science were elaborated between 2017-2018. These healthy subjects was chosen from a common blood donor who had not infected and exposed to chemical materials, different rays, reagent substances, drugs or other that could hasard mitotic alteration percentage.

In one sample, 0.5 ml to 1.0 ml Peripheral Blood were taken and manipulating selecting; (a) 48–h stimulated culture test and (b) 72 h were stimulated culture. The cultures were set up here after a modified method. For culture , 3-5 × 106 cells will culture in 4 ml tissue culture media (Gibco-BRL Grand Island, RPMI 1640, NY,USA) added with 10-14 percent inactivated with heat of fetal calf serum (Gibco-BRL Grand Island, RPMI 1640, NY,USA) at 370C in five percent CO2 condition. The standard of metaphases cell growing from 48-hours stimulations as well as from 72-hours cultures were noted base on references with minor modified method. Finally, the cultured lymphocytes will treat with Colchicine (Grand Island, NY, USA Gibco-BRL)total volume, 10 µg/ml then will incubate at 37oC for an with three minutes. The volume of the culture flask then centrifuge for 8 minutes at rpm 1000 in 10 ml of 75 KCl mM (0.55%) re-suspend and pre-warmed to 37oC for twenty minutes. In this step, one ml of standard Fixative (1:3 acetic acid: methanol) was increased in to the flask, and the step of fixation were done 4 times. Twenty slides were arranged for every sample test and stained for three minutes with Giemsa stain. Examination of slides were tested with an Nikon light microscope. Hundred spread of metaphases was evaluated for all metaphases. Metaphases were defined based on ISCN[17].

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[18].

Statistical Analysis

The program Package of statistical for research work v.21.0 for Windows (Chicago, IL) was used for the statistical analysis. Differences in mitotic index frequencies between the two period cultured with comparison by using Chi-square and Fisher's exact tests.

Results

Observation of mitotic activities and chromosome index in lymphocytes of donors

peripheral blood have been utilized to evaluate the 48-h and 72-h effect of similar culture concentrations noticed in the present experiment.

A brief study of the chromosome indices and karyotype datas is exhibited in Table 1. As resulted the chromosome index of the harvested cultures at seventy two hours was higher significantly than the forty eight hours 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 were evaluated among blood volunteers.

The culture conditions were equal for both harvesting times. The comparison of mitotic of each volunteer to respond to PHA stimulation and the mitotic index availability, in a results of blood donors, of mitotic populations were noticed in a different culture frequency. Also no qualitative difference were set up between the two types of cultures, observed in both cultures period. Overall, in the present results a herald higher in metaphase index of all blood donor was investigated. However, many mitotic available an higher degree of metaphases contraction. A non-random selection of well

Table 1. Mitotic indices of cultured for 48h 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% ^{a*}	
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	

a^* ; Significantly different from 48-h frequency $^*P \leq 0.05$

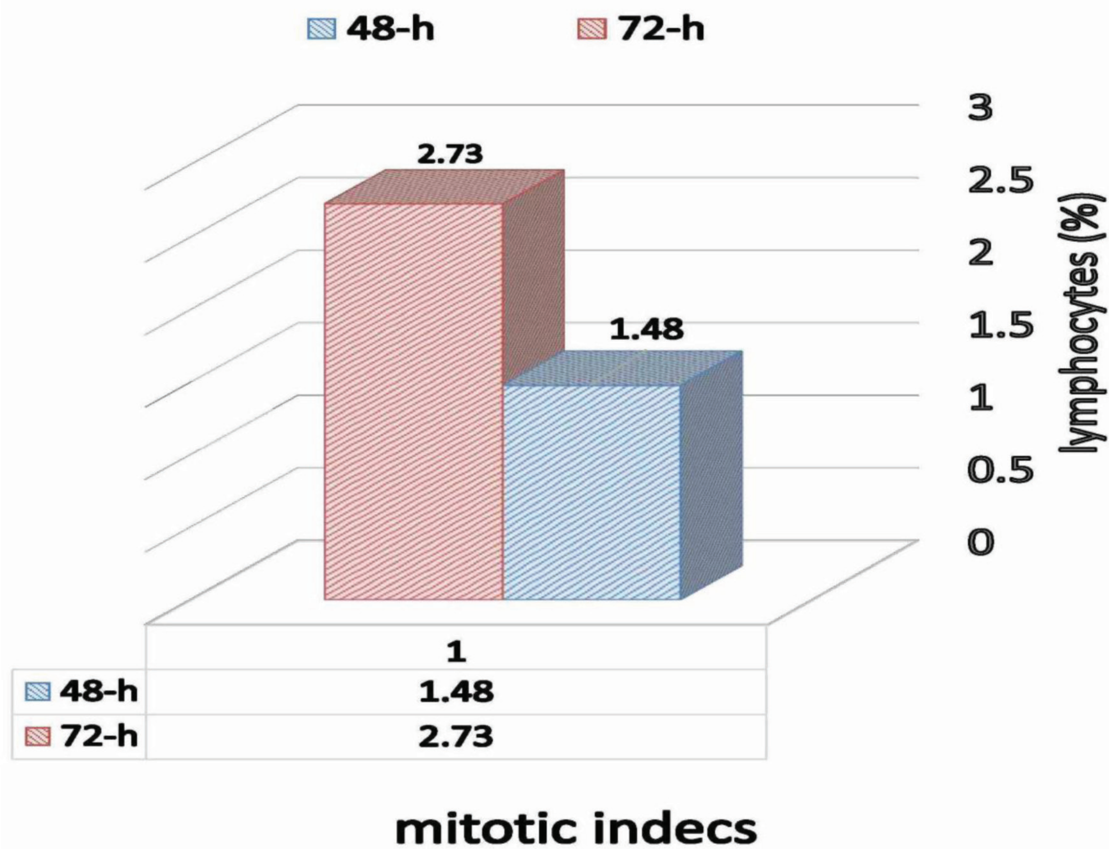

Figure 1. Comparison of Frequency of Normal Human Lymphocytes Cell Cultured in 48- h and 72-h.

prepared slides including cell populations index were taken place 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.

Discussion

It was considered that the forty eight –hours cultures consist of division first mitosis. In other word, as many as of geneticist has used seventy two hours as the ideal culture period.

Most researchers in the laboratories investigate karyotypes, prepared from mitotic cells that have been treated Phytohaemagglutinin (PHA) stimulus for 72-h in the prometaphase or metaphase stage of the cycle cell, when chromosome consider their most condensed performances[5,8,10]. It would be prudent to take advantage of higher metaphase indices of cultures and treated with PHA at seventy two -hours[18]. In an accordance with reports in the current literature and the result of this project performed on all healthy blood donors as described earlier presented here, the metaphases index of the PHA treated cultures and harvested at seventy two hours was higher significantly than the forty eight-hours samples (2.73% at 72 h vs. 1.48% at 48 h, $p \leq 0.05$). On the other hand, the metaphases index and kinetic cell cycle of untreated metaphases were noticed to different higher for normal samples was reported[20]. The lymphocytes sub-population 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. Therefor, any differences in the cell growing percent 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[11]. In spite of the lymphocyte kinetic findings, lymphocytes growth revealed on cultures incubates for less than forty eight

hours can not be possible owing to the less of an average count of mitotic indexes[21,22]. Evaluation of the percentage of chromosome structural abnormalities (CA) in different culture period mostly affected by radiation for circular metaphases is now currently manipulated in karyotyping evaluated on human individuals[18]. According on the results reported by some workers, it is reasonable to express that forty eight hours can not be the difficult period in the detecting of spontaneously happening chromosomal abnormalities in healthy normal volunteers. Despite the possibility that as many as abnormal lymphocytes may be eliminated or that many abnormalities might be restituted from fort eight- to seventy two -hour gap, the results reminds that a seventy two hours culture does not influence significantly the spontaneous karyotype results in this field. Perhaps, the cell proliferating populations at the forty eight- and seventy two –hours 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 seventy two hours[18]. In other word, as many as the chromatid and derived abnormal metaphases would be higher if the lymphocytes growth through 2 or increase cell cycles growth[23-25]. In spite of the lymphocyte kinetic findings. Karyotyping research performed on harvest incubated for less than forty eight- hours can not be possible owing to the deficit of an available number of chromosome indexes[26,18].

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 of this proposal.

Acknowledgments

Funding: We, hereby, would like to express our gratitude to the Research Council of Shahid Beheshti University of Medical Sciences, Tehran, Iran. for their financial support of this project (Grant number: IR.SBMU.RETECH.REC.1397.558).

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Studies are Institutional Review Board approved and reviewed annually and have been registered with the National Institutes of Health: IR.SBMU.RETECH.REC.1397.558. Written informed consent was obtained with each blood donor receiving careful discussion of potential risks, benefits and the experimental nature of the procedure.

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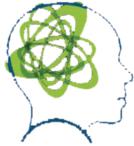

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