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Original Research Article

EFFICACY OF IMMEDIATE, 24 HOURS VERSUS 48 HOURS CULTURE METHOD IN CYTOGENETIC STUDY OF PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA

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ABSTRACT

Background: Leukemia is the most common childhood cancer in India with relative proportion varying between 25 and 40%. 60 to 85% of all leukemias reported are acute lymphoblastic leukemia (ALL).Karyotyping is a technique that provides critical diagnostic and prognostic information that allows the delivery of appropriate treatment. Success rate depends on sample conditions, type of disease and culture conditions.

Aim: To compare efficacy of culture methods at varying times in karyotyping for genetic abnormalities of Acute Lymphoblastic Leukemia.

Materials and Methods: Bone marrow aspirates (0.6 ml) after adding 15 ml RPMI medium were divided into three parts for immediate(direct) culture, 24 hour culture and 48 hour culture method , were incubated according to their respective time duration and karyotyping was done.

Result: In immediate culture, there were very few small sized cells with no metaphases. In 24 hour culture, cells had a moderate population with no analysable metaphases. In 48 hour culture, cell population was high with bigger sized cells and 20 or more metaphases were observed per slide.

Conclusion: 48 hours culture method is better than immediate and 24 hour culture in ALL patients.

KEY WORDS: Acute Lymphoblastic Leukemia (ALL), Bone Marrow, Karyotyping, Culture.

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INTRODUCTION

Childhood leukemia accounts for more than 40% of new childhood cancer cases. Karyotyping of cytogenetic abnormalities in such cases continues to provide critical prognostic information which allows delivery of appropriate intensity of treatment. Many factors contribute to the poor morphology of chromosomes in karyotyping. External factors include the culture medium used, protocol used in chromosome banding, sample drawing procedures and travel time. Internal factors include the poor inherent viability of leukemia samples. Leukemic cells have poor viability and tend to die more quickly than normal cells [1]. In patients with leukemic disorders , chromosome analysis for bone marrow cells for diagnostic purposes is hampered by the low yield of mitoses, the poor spreading of the

metaphases and the blurred aspect of chromosomes [2]. The belief that selective processes may occur during the culture of bone marrow cells has long deterred the use of cell culture technique as an aid to diagnosis of leukemia [3]. There is need for better understanding of which culture duration is superior considering the fact that cytogenetic analysis of leukemic cells is often constrained by limited amount of bone marrow sample available. Such studies have been reported in western literature but not in Indian population.

MATERIALS AND METHODS

Bone marrow aspirates taken from iliac crest were collected from 20 haematologically confirmed pediatric cases of Acute Lymphoblastic Leukemia after obtaining ethical clearance from ethics committee of Maulana Azad Medical College and associated Lok Nayak hospital. The patients were recruited from pediatrics ward of Lok nayak hospital. Bone marrow aspirates (0.6 ml) after adding RPMI medium(15ml) were divided into three equal parts for 1.) immediate(Direct) culture 2.) 24 hour culture and 3.) 48 hour culture method. For direct culture method samples were incubated for 1 to 2 hours. For 24 hour culture and 48 hour culture samples were incubated at 37^0C for 24 hour and 48 hour respectively. After incubation 0.05 ml of colchicine was added. Centrifugation was done at 1000 rpm for 10 minutes. Supernatant was discarded. 5 ml of hypotonic solution was added to the pellet (0.075M KCl prepared by adding 0.56 gm in 100 ml distilled water) and incubated for 45 minutes at 37^0C. Centrifugation was done at 1000 rpm for 10 minutes. 5 ml of chilled fixative was added (methanol: glacial acetic acid in the ratio 3:1). Again centrifugation was done at 1000 rpm for 10 minutes. This was repeated till pellet turned white. Suspension was dropped from a height of 40 cm on a chilled glass slide. Slides were air dried and labelled with trypsin. Each metaphase spread was screened for well banded metaphase spreads. At least 10 recordable and analysable meta-phase spreads were screened and photographed.

RESULTS AND DISCUSSION

Out of the 20 cases seen results were obtained in 14 cases. In immediate culture there were very few small sized cells with no metaphases. In 24 hour culture cells had a moderate population with no analysable metaphases. In 48 hour culture cell population was high with big sized cells and 20 or more metaphase spreads were observed per slide. Number of metaphases increased with culture time duration with peak reaching at 48 hours. (Figure 1,2,3).

Fig. 1: Slides showing comparative population, size and metaphases in immediate , 24 hour culture and 48 hour culture methods . Population and size of cells of cells are maximum in 48 hour culture method. Metaphases are seen only in 48 hour method.

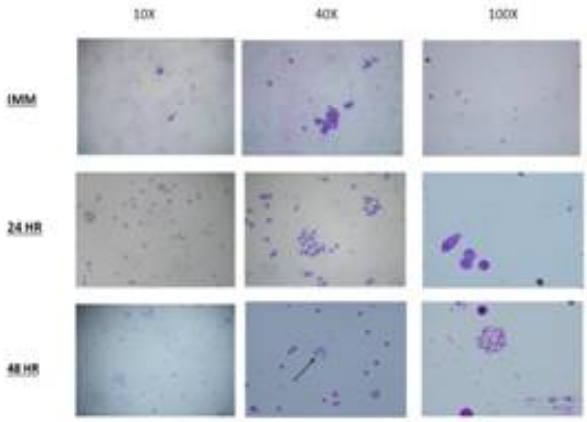


Fig. 2: Showing relative cell population, cell size and number of metaphases in immediate, 24 hour and 48 hour culture methods.

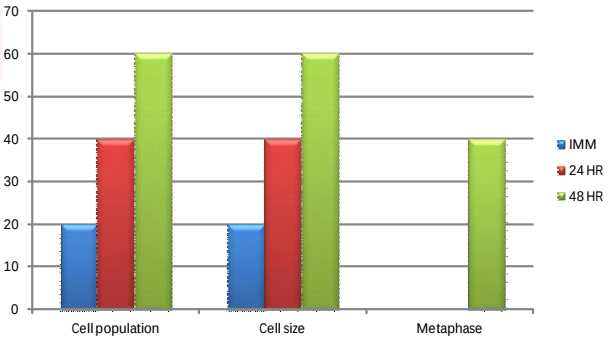
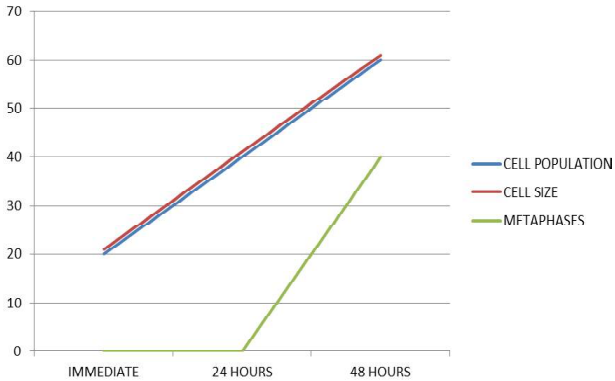


Fig. 3: Showing cell population, cell size and number of analysable metaphases per slide in immediate, 24 hour culture and 48 hour culture methods.



Acute lymphoblastic leukemia (ALL) is characterized by clonal proliferation, accumulation, and tissue infiltration of neoplastic cells. They are mainly regarded as childhood diseases, with an early incidence peak at two to five years of age, where they represent about 80% of the childhood leukemia in the United States, and occur with an incidence of 30 cases per one million population per year. [4] In India, incidence of ALL is one fourth of all childhood cancers and three fourth of all childhood leukemias [5].

Karyotyping is a technique that provides critical diagnostic and prognostic information that allows delivery of appropriate treatment. This technique solely depends on tissue culture success and availability of metaphases. Use of chromosome banding techniques has provided valuable diagnostic tool in various malignancies. The application of these methods however is often restricted due to low yield of mitotic cells and patients' unwillingness to comply with repeated bone marrow aspiration. The success rate depends on sample conditions, type of disease and culture condition. The success rate varies from lab to lab from 77.6 - 90% and 87% as reported by Heng et al and Ito et al respectively [6,7].

The chromosomes of leukemic cells were first studied from cultured bone marrow or blood cells. Soon afterward, direct method of metaphase preparation which avoided cell culture were developed and widely adopted. These methods were believed to give a more accurate result because they avoided possibility of the differential proliferation of bone marrow cell populations during culture, and particularly the overgrowth of leukemic cells by normal bone marrow cells. It now appears that this belief was not fully tested [3].

Malekasgar et al in their study on 25 pediatric patients which compared four different culture methods , found that direct method was more efficient for ploidy analysis [8]. According to Gordon et al chances of finding a chromosomally abnormal clone in their study were about the same with any of three methods that is direct, 24 hour and mitotic synchronisation methods [9].

Reports from patients with leukemia (Berger et al and Knuutila et al) indicate that karyotypically abnormal cell lines are detected more accurately in cultured than in directly prepared bone marrow [10,11].

Reports from patients with acute promyelocytic leukemia (Berger et al) and a patient with refractory idiopathic sideroblastic leukemia(Knutilla et al) indicate that karyotypically abnormal cell lines are detected more accurately in cultured than in directly prepared bone marrow [9,10]. A further study has shown this also to be the case in chronic myeloid leukemia and in some acute leukemias and preleukemic conditions [12].

Fitzgerald et al found cultured cells to be more reliable indicators of clonal cytogenetic abnormality, but the results may be different for different forms of leukemia. In their study on 22 AML patients they found 24 hour culture of bone marrow from patients with AML and APML gave more analysable metaphase cells and improved chromosome morphology compared with direct preparations. Culture increased the proportion of cytogenetically abnormal cells, and in 6 bone marrows were direct preparation failed, a result was obtained from the cultured preparation [3].

Sakari knutilla et al in their study on short-term culture on the type and frequency of chromosomal aberrations found in bone marrow cells from patients with hematologic disease compared classic direct method (1 -2 hr incubation) with methods involving culture for 1 -4 days. Culture yielded a higher proportion of cells belonging to the abnormal than to the normal clone. The phenomenon occurred in chronic myeloid leukemia, myeloid and acute lymphoid leukemia, and preleukemic conditions. They implied that to improve the chances of detecting cytogenetically abnormal cells bone marrow cells should be cultured. They reported that the proportion of cells belonging to abnormal clones among bone marrow cells from patients with leukemia or preleukemia is significantly higher in preparations made after culture for I -2 days than in those made by the so-called direct method (I -2 hr). Bone marrow samples from patients with leukemia, suspected leukemia, or preleukemia were used to elucidate the relative proportions of cells belonging to cytogenetically different clones in preparations obtained by the

"direct" and "culture" methods [12].

In our study all the analysable metaphases were found in 48 hour culture. Cell size and cell number increased with culture duration. Our study demonstrates that increasing the culture time maximises the chances of obtaining analysable metaphases in pediatric Acute Lymphoblastic Leukemia. Findings in our study are similar to those reported by Knutilla, Berger and Fitzgerald et al and dissimilar from those observed by Gordon et al and Malekasgar et al. (Fig. 1, 2 and 3).

CONCLUSION

We suggest that in routine practice for cytogenetic diagnostic study of ALL, 48 hours culture method is better than immediate and 24 hour culture. This study will help decide the best culture duration for cytogenetic analysis of ALL in India since bone marrow samples procured are often limited in amount due to difficulty in aspiration especially in children.

ABBREVIATIONS

ALL- Acute Lymphoblastic Leukemia

RPMI- Royal Park Memorial Institute

Rpm- rotation per minute

AML- Acute Myeloid Leukemia

APML- Acute promyelocytic leukemia.

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Conflicts of Interests: None

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