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ALDH Expression in Hematopoietic Stem Cells Derived from Cord Blood: Effect of Transfer Time

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Abstract: The human aldehyde dehydrogenase (ALDH) activity measurements have recently been considered a quick and accurate quality control test used to determine the function of cord blood cells. The relationship between high ALDH activity of hematopoietic stem cells and engraftment is also known. However, there is limited data on the relationship between ALDH expression and transfer time of the cord blood to the laboratory in cord blood banking.

The aim of this study is to investigate whether the transfer time has an effect on ALDH expression. 20 volunteers were included in the study. After collection of the cord blood, transfer times to the laboratory were calculated. Subsequently, CD34+ cell count, Total Nucleated Cell (TNC) count, and ALDH expression were analyzed. ALDH expression was found to be high in cord blood containing a high number of CD34+ cells. Similarly, a positive correlation was detected between TNC count and ALDH expression. There was no correlation between the transfer time, which is an important parameter in cord blood banking, and ALDH expression.

The findings of the present study show that ALDH test can be used in cord blood banking, and it reveals for the first time that it can be used safely regardless of the transfer time.

Keywords: ALDH; Cord Blood; Hematopoietic Stem Cell.

1. Introduction

The human aldehyde dehydrogenase (ALDH) superfamily consists of 19 known functional genes. It is classified into 11 families and 4 subfamilies in different chromosomal settlements (1-2). ALDH enzymes can also be found in cytosol, nucleus, mitochondria, and endoplasmic reticulum. Enzyme levels of ALDHs may vary in human tissues and organs depending on the enzyme family and subfamily (3). ALDH has been defined as an important enzyme for preserving normal hematopoietic stem cells (4) and is used as a marker to identify and isolate various types of stem cells (5-6). Stem cells are defined as cells that are capable of self-renewal and differentiation into mature cells that form certain tissues and organs (7). Since stem cells are usually found in small numbers in tissues and organs, different strategies are needed at the stages of their isolation and enrichment. The presence of single or multiple biomarkers is important for their use in both research and therapeutic areas (8). Cord blood is a very rich source of hematopoietic stem cells. It has been successfully used as a source of hematopoietic stem cell in bone marrow transplantation since 1988. The number of transplants performed has exceeded 35.000 units (9). Collection and transfer of cord blood are the major limitations. Unfortunately, not all blood collected can be used in hematopoietic stem cell transplantation and/or regenerative and reparative medicine applications.

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One of the most important reasons for this is bacterial or fungal infection (10) and the presence of too few cells due to the inadequate volume of blood collected during the blood collection stage (11). In addition, factors such as the storage method, loss of cells during freezing and thawing, and exposure to temporary warming determine the quality of cord blood (12). Recently, ALDH activity measurements have been considered a quick and accurate quality control test used to determine the function of cord blood cells (13-14) The relationship between high ALDH activity of hematopoietic stem cells and engraftment is also known (15) However, there is limited data on the relationship between ALDH expression and the transfer time of the cord blood to the laboratory in cord blood banking. The aim of this study is to investigate whether the transfer time has an effect on ALDH expression.

2. Material and Methods

2.1. Study Population

Approval was obtained from the Ethics Committee of Akdeniz University Faculty of Medicine (Decision No: 147, Date 21.02.2018) for the study. After the approval was obtained, families who agreed to participate in the study among those who applied to Akdeniz University Technopark Babylife Cord Blood and Human Cell-tissue production center were included in the study. Samples were taken from 20 cord blood units for the study. The remaining parts of the samples were used, and no additional samples were taken for the quality control tests.

2.2. Cord Blood Collection

Cord blood was collected from the umbilical vein. Before collecting the blood, the area was cleaned with a disinfectant containing iodine. Cord blood was then taken into the blood collection bag containing CPDA. Collection time and reception time at the laboratory were recorded. The transfer time of blood was calculated using these data.

2.3. Total Nucleated Cell (TNC) count

TNC count was carried out with a fully automatic cell counting (Swelab alpha-no-111-450) device in our laboratory.

2.4. CD34 Count

CD34 cell count was carried out according to ISHAGE protocol (16). Live cells were detected by 7-AAD (BD Pharmingen 7-AAD- 559925), and CD34 (mouse anti-human CD34 -345802 BD) and CD45 (mouse anti-human CD45-345808 BD) monoclonal antibodies were used. Flow cytometric analyses were performed with BD Facs Calibur.

2.5. ALDH Assay

Aldehyde dehydrogenase (ALDH) assays (AldeRed™ ALDH Detection Assay SCR150 Sigma-Aldrich) were performed according to the manufacturer's instructions. After the samples were prepared, flow cytometric analysis was performed (with the BD Accuri C6 flow cytometer). ALDH values were calculated as Mean Fluorescence Intensity (MFI).

2.6. Statistically Analysis

SPSS 21 software was used. Mann–Whitney U test and Pearson correlation test were carried out. $P < 0.05$ was considered statistically significant

3. Results

When the relationship between CD34+ cell counts and ALDH expressions of cord blood samples included in the study was evaluated, ALDH expression of samples containing a high number of CD34+ cells was also found to be high (Figures 1, 2).

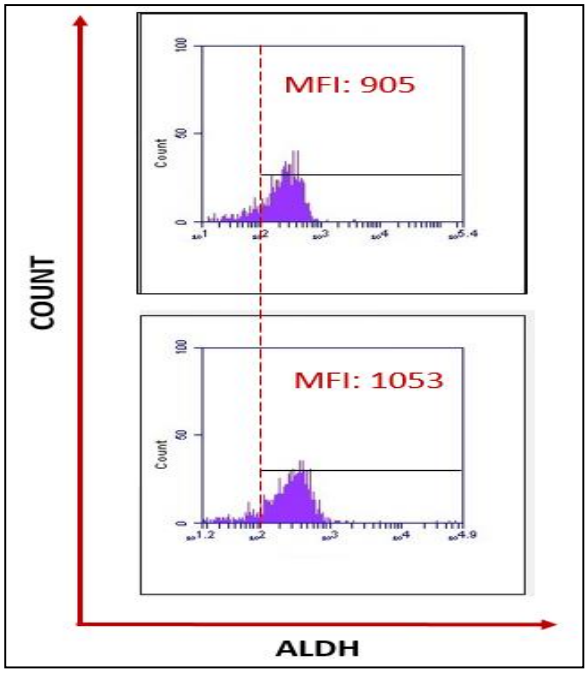

Figure 1: ALDH Flow Cytometry Histogram. Comparison of a sample containing a low CD34+ cell count with a sample containing a high CD34+ cell count. (MFI: Mean Fluorescence Intensity n=20 p<0.05 value according to the Mann-Whitney U test).

When the relationship between the total nucleated cell count and ALDH expression was examined, samples containing high TNC count were also found to have high ALDH expression (Figure 3). In order to determine whether ALDH expression was affected by transfer time, the relationship between the ALDH expression levels and transfer times of samples that reached the laboratory within different periods of time was examined. Although CD34+ cell count showed a positive correlation with TNC count, it was found to have no correlation with transfer time (Figure 4). This result revealed that ALDH can be safely used as a cellular quality control parameter in autologous cord blood banking without being affected by transfer time.

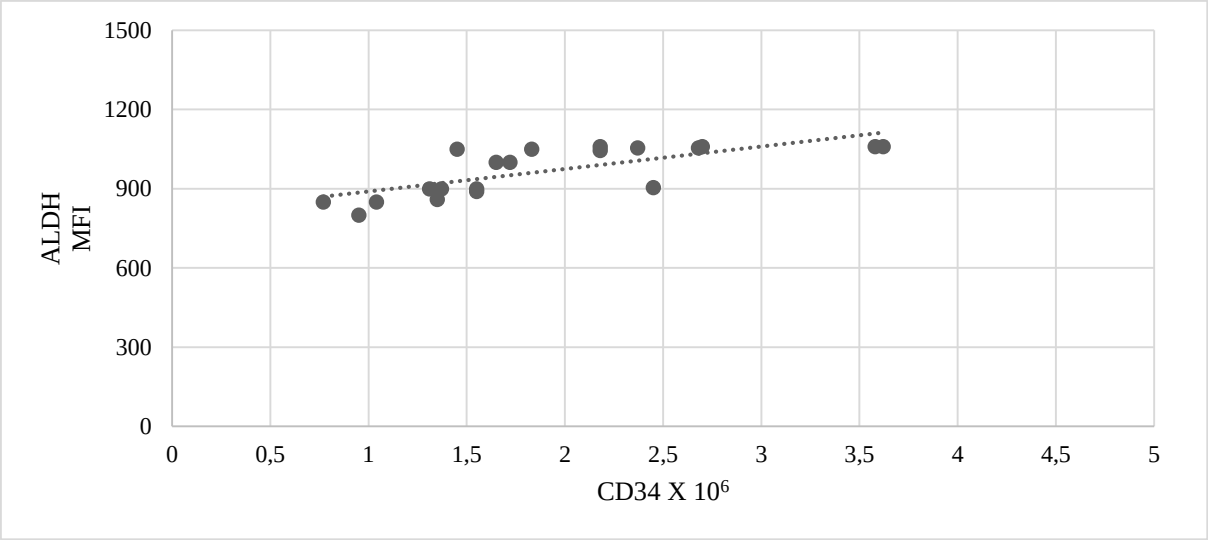

Figure 2: The relationship between ALDH expression and the CD34+ cell count (MFI: Mean Fluorescence Intensity n=20 $p<0.05$ value according to the Pearson correlation test).

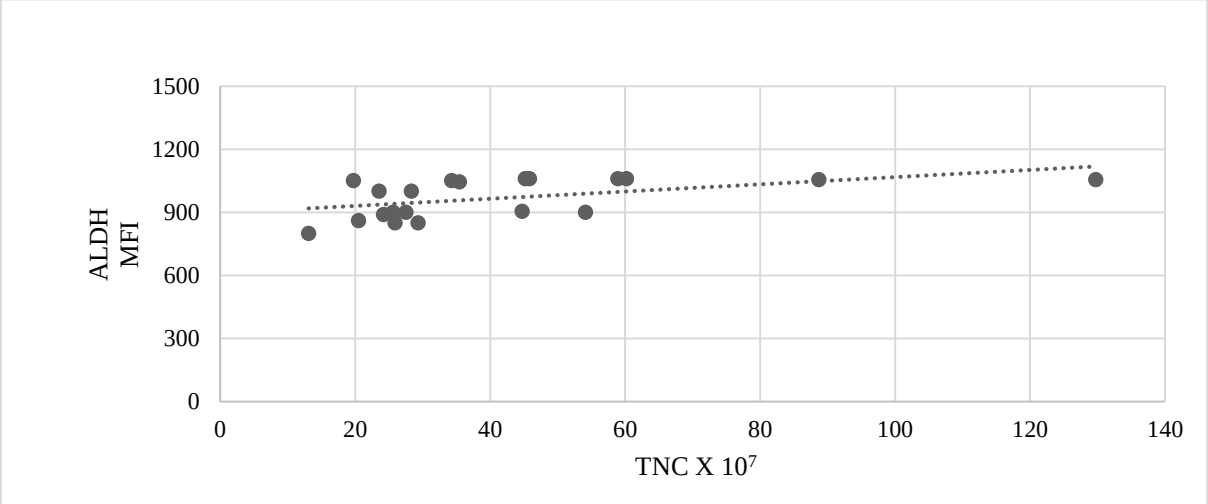

Figure 3: The relationship between ALDH expression and TNC count (MFI: Mean Fluorescence Intensity, TNC: Total Nucleated Cell, n=20 $p<0.05$ value according to the Pearson correlation test).

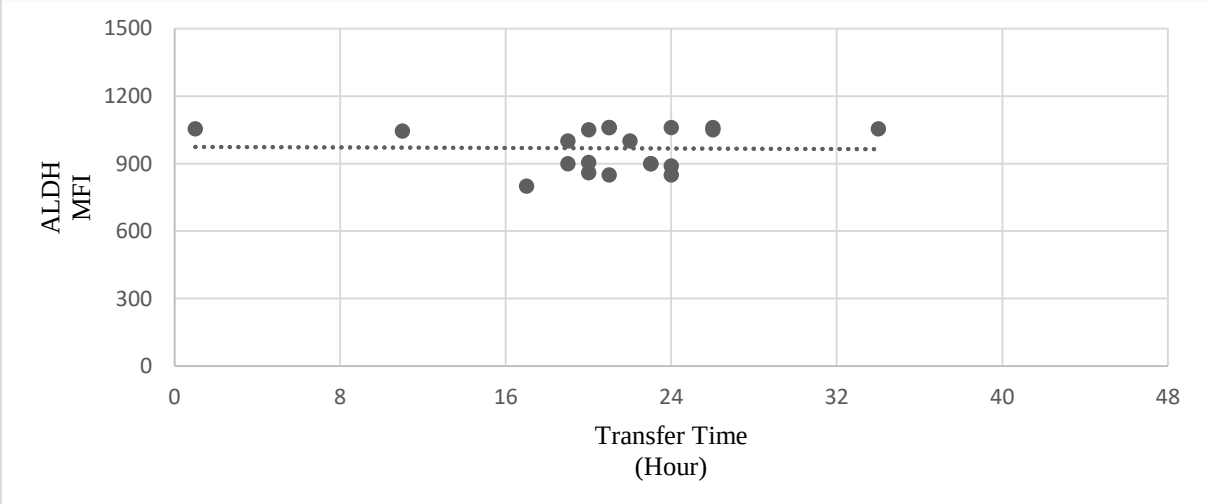

Figure 4: The relationship between ALDH expression and transfer time (MFI: Mean Fluorescence Intensity n=20 $p>0.05$ value according to the Pearson correlation test).

4. Discussion

Hematopoietic stem cells ensure maintenance of the blood tissue under physiological conditions and bone marrow restructuring after bone marrow transplantation (17). It uses its two important features when performing these functions: self-renewal and differentiation (18-19). The cord blood banking system enables the hematopoietic stem cell to acquire the characteristics of a ready-to-use product with a shelf life. Although cord blood is an important source, due to the low number of stem cells contained in it, issues such as delayed engraftment and primary graft failure may occur after transplantation (20). In such cases, quick and reliable tests to assess the potency and quality control parameters of stem cells contained in cord blood both before freezing and at the time of thawing for transplantation are even more important. Today, the number of granulocyte-macrophage colony-forming unit (CFU-GM) is the best indicator of neutrophil engraftment and overall survival of the recipient (21). However, colony-forming unit (CFU) testing is expensive, time-consuming, and difficult to standardize. Due to these characteristics of CFU, there is an increasing need for alternative approaches. An alternative and promising approach is the analysis of ALDH, which is based on increased enzyme activity in hematopoietic stem cells (22). It has been revealed that ALDH can also be used in cord blood banking, and particularly, it remains reliable after freezing-thawing (23). In the present study, the time needed to deliver the blood to the laboratory after it is collected, which is an important restrictive factor in cord blood banking, and the effect of this period of time on ALDH expression was examined. ALDH expression was determined flow cytometrically in these samples obtained from 20 units of cord blood in total. A positive correlation was found between CD34+ cell count and TNC count and ALDH expression in support of the literature. However, it has been found that the transfer time does not have a negative effect on ALDH expression. The cord blood samples included in the study were selected from blood samples collected within the first 48 hours in accordance with the Cord Blood Banking Regulation published by the Ministry of Health of the Republic of Turkey.

5. Conclusions

The data we obtained support that ALDH test can be used in cord blood banking and reveal for the first time that it can be used safely regardless of the transfer time.

Conflict of Interests

The authors report no conflicts of interest

Financial Support

None

Author Contributions

D.B: Designed and performed experiments, analysed data and wrote the manuscript.

Ethical Approval

Approval was obtained from the Ethics Committee of Akdeniz University Faculty of Medicine (Decision No: 147, Date 21.02.2018) for the study.

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