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RESEARCH ARTICLE

STUDY OF BLOOD STORAGE EFFECTS ON NEUTROPHILS TOTAL COUNT, PHAGOCYTIC ACTIVITY AND MORPHOLOGY

Dr.WeamSaad Al-Hamadany
Dean Assistant for Scientific Affairs/ College of Pharmacy/ University of Al-Muthana, Iraq.

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Corresponding Author

Dr.WeamSaad Al-Hamadany

Abstract

Transfusion of stored blood faces many problems, and Neutrophils retardation is one of these. A total of ten human blood samples were collected and used upon this study to investigate storage effects on Neutrophils total count, phagocytic activity and morphology. The results showed deterioration in these parameters after two days; 48 hours of storing, due to apoptosis induction. Conclusion: Upon storing whole blood at 4°C, apoptosis, total count and phagocytic activity retardation can be steady up to 24 hours, without adding any materials for preservation or cytokines, otherwise retardation of these parameters will be significant after 36 hours.

Introduction

Storage is one of the problems facing successful transfusion since whole blood suffer from deteriorate over time. Problems associated with storage can include lyses of RBCs, loss of complement; change of pH, decrease antibody potency and our subject under focus which is WBCs apoptosis induction pointing to Neutrophils. Neutrophils can survive for days during storage (Lewis, 2006); but the question is how far they will stay active and functional within accepted values. When Neutrophils starts the Caspase serial events of apoptosis (programed cell death), they will suffer from suppression in activity, decrease in count and several morphological changes. The aim of this study is to determine the time post collection of blood that Neutrophils can be considered as effective cells with accepted count and activity.

MATERIALS and METHODS

- **Blood Samples:**

Ten human blood samples of (10 ml) freshly collected from adult males, nonsmokers with no clinical signs. Blood collection was done using heparinized tubes and mixed gently; storage conditions were the standards; under 4°C in refrigerator as recommended by (Lewis *et al.*, 2006).This study was accomplished between; Feb. 2014 to Apr. 2014.

- **Tests applied on blood samples:**

The following tests were applied on the ten blood samples at the times; zero (immediately after collection), one, six, 24 and 48 hours after collection blood samples.

☒ **NBT Test; Nitro-blue Tetrazolium Test:**

This test was used to estimate the phagocytic activity of Neutrophils and to detect any change in these cells activity through repeating this test during several periods of storage. Active Neutrophils are able to reduce Nitro-Blue Tetrazolium (NBT) dye,(Sigma, St Louis, MO, USA), using respiratory oxidative burst, and forming (Formazan particles). These particles deposit as dark blue crystals, hence called positive cells, (Al-Hamadany,2011).

☒ **Total Neutrophils count:**

This parameter was used to detect any drop in Neutrophils count, and accomplished using Digital Blood Hemolyzer Instrument, produced by BioTek.Comp(USA).

☒ **Detection of Neutrophils Morphologicalchange:**

Neutrophils morphological change was detected by examination of blood films preparations. Smears were stained using Leishman's stain (Fluka comp., Germany) and were examined under both 60X magnification and oil immersion 100Xmagnification using light microscope.

- **Statistical Analysis:**

Mean calculations was the only statistical test applied on this research results, mean values were calculated for Neutrophils counts and positive phagocytic cells percentages for the ten blood samples results and for each testing time data.

RESULTS

`The table (1) shows all obtained results for total Neutrophils counts and phagocytic activity percentages in relation to storage time of tests performed; each value represents the mean value of the ten blood samples results.

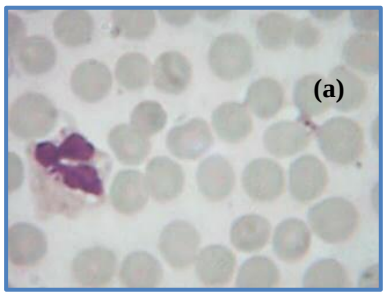
There was no sharp drop in Neutrophils count during the first 24 hours along trial, the counts decreased then, and active phagocytic Neutrophils relative number dropped after 24 hours of storage obviously.

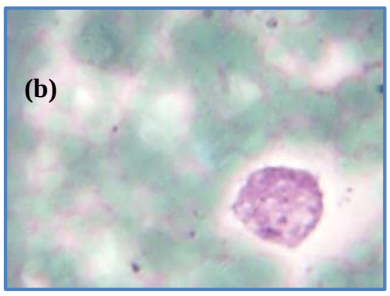
There were morphological changes in Neutrophils during examination of prepared smears; these changes reached the maximum after seven days of storage in most blood samples involved in this study with a different intensity. The recorded changes were: nuclear matters degradation, abnormal nucleus texture, abnormal staining ability of cells and nuclei, abnormal cellular membranes, different stages of apoptosis, swollen Neutrophils. Identifications depended on (Theml *et al*., 2004), Picture (1).

Table (1): Results obtained for total Neutrophils counts and phagocytic activity percentages in relation to storage time of tests performed.

Order of reads	Time	Active Neutrophils (positive cells %)	Neutrophils count x 10^9/L
1	Zero	78*	4.1*
2	1 hr.	76	3.8
3	6 hr.	67.5	3.5
4	24 hr.	59	3.1
5	48 hr.	42	2.8

*:Each value represents the mean value calculated for the obtained results of the ten blood samples.





Picture (1): Heparinized Blood film under 100Xmagnification stained with Lehman's stain. (a):Normal Neutrophil before blood Storage; (b): Apoptotic Neutrophil after blood Storage (7 days); shows degradation of nucleus.

DISCUSSION

This study concerned with storage effects on Neutrophils total number, active phagocytic relative number and detection of morphological changes occurrence as an evidence for apoptosis.

Firstly, heparin was used in this trial since it does not alter blood cells size and it is the best anticoagulant for cells activity testing as the opinion of (Bain *et al.*, 2012 and Lewis, 2006). The best laboratory techniques to preserve leucocytes viability in whole blood specimens for subsequent tests is to use heparin as anticoagulant with nutrient medium addition and store at 4°C as reported by (Hodge *et al.*, 1999).

On the level of phagocytic activity, relative number of active Neutrophils or positive to NBT test along trial had decreased obviously in relation to storage time, and reached unaccepted levels at time 48 hours, whereas, nearly half of the active Neutrophils became inactive.

Price and Dale, (1977) stated that; the most feasible temperature for peripheral Neutrophils storage is 4°C rather than room temperature since cells under such conditions can survive and maintain their function for a few days.

VanRaam *et al.*, (2008) reported that despite of the fact that Neutrophils have a very short life span in peripheral blood after leaving bone marrow; *in vitro* analysis showed that these cells can survive until 12 hour before inducing apoptosis. And they explained these outcomes due to the presence of Granulocyte Colony-Stimulating Factor (G-CSF), which is a cytokine able to inhibit apoptosis in these cells. Polymorphnuclear cells (PMNs) including Neutrophils can survive upon storage time to 21 days until apoptosis is evident as stated by (Biffl *et al*, 2001).

Drewniak *et al*., (2008) concluded upon their experiment that granulocyte concentrates can be stored without loss of *in vitro* viability and functionality for at least 24 hours; and *in vivo*, granulocyte transfusions may be an effective therapy for neutropenic pediatric patients suffering from life-threatening infections.

The scientists Klebanoff and his colleagues (1992); had tested the use of Gamma-Interferon (IFN-γ), a cytokine to prevent the fast decline in stored Neutrophils activity. While (Price &Dale,1977), gave a standard base for Neutrophils storage by keeping them under 4°Ctemperature and proved how they are able to restore their function *in vivo* even after storage for a few days in these conditions, hence the best temperature to store Neutrophils is 4°C to delay apoptosis induction and function retardation.

From another view, stored blood has limited oxygen demand, and since the phagocytic activity of Neutrophils mostly oxidative burst, this function can be limited in stored blood since there is no supply of oxygen. This opinion is consistent with the results of (Muniz-Junqueira *et al.* 2004).

Neutrophils lose chemotaxis firstly; followed by microbial killing then phagocytosis and these losses were irreversible (Glasser, 1977). Also he recommended that storage for Neutrophils should be limited to 24 hrs. , because sever defects occur between the first and second day.

Changes in blood cells morphology occur in short time storage, as stated by (Mckenzie, 2004). Apoptosis in Neutrophils was described broadly by (Kuijpers*et al.*, 2003) and his team upon their research, they identified apoptotic Neutrophils depending on several toxic changes due to storage. And restriction of phagocytic activity *in vivo* and *in vitro;* also was investigated.

Patrone *et al*., (1979), found that storage for PMNs at 4^{o} C caused loss of function detected by NBT test estimation within 48 hrs. Hammer *et al.*, (1986), concluded that storage of heparinized blood for 48 hrs. at 4^{o} C caused decrease in phagocytes function. That was in agreement with our results. Baca *et al.*(2006) demonstrated that storage not more than 4 days can keep steady many hematological parameters of whole blood.

The researchers (Parvathenani *et al.*, 1998) and his colleagues recommended using cAMP to delay Apoptosis caspase reactions in stored human Neutrophils and keeping them viable. Apoptotic morphology features can rise in Neutrophils when assessed at 12, 24 and 48 hrs; but these events can be inhibited by using glucocorticoids and hydrocortisones and save the survival of these cells by delaying apoptosis(Liles *et al.*,1995).

Finally **(**Bain*et al.*, 2012), in the text referenced book of hematology, explained all the morphological changes of stored blood cells in expand, they stated that the changes are retarded but not abolished in blood stored at 4^{o} C. they mentioned that total Neutrophils count suffer from diminishing while storage undergoing due to phagocytosis of apoptotic cells by the remaining active Neutrophils.

CONCLOSION

Upon storing whole blood at 4°C, apoptosis, total count and phagocytic activity retardation can be steady up to 24 hrs, without adding any materials for preservation or cytokines, otherwise retardation of these parameters will be significant after 36 hrs.

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