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Lipid Peroxidation Andascorbic Acid Concentration During blood Storage

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Abstract : Storage of donor blood for transfusion is a critical issue in blood transfusion practice. Biochemical changes attendant on storage of donor blood have been variously documented and are associated with reduced viability of transfused red cells. Lipid peroxidation as a result of oxygen free radicals and reactive oxygen species may be additional storage lesions which are not yet attracting attention of many blood banks. This investigation considered the possible peroxidation of red cell membrane and ascorbic acid concentration during storage. Blood samples were collected from twenty apparently healthy bloods donor at the University of Nigeria Teaching Hospital, Enugu, Nigeria using CPDA anticoagulant solutions. The blood samples were subsequently divided into blood storage bottles and lipid peroxidation measured as malondialdehyde (MDA). Ascorbic acid concentration wasalso determined on the first day of collection and subsequently after two, four and six weeks at 4-6^{o}c. Lipid peroxidation products measured as MDA increased progressively with significant differences between concentrations of the first day of collection and second, fourth and sixth weeks of storage ($P<0.05$). However, there was no significant difference ($P>0.05$) between the ascorbic acid of the first day of collection (1.27± 0.06) and the second week of storage (1.16±0.39).Lipid peroxidation increased with storage while ascorbic acid concentration decreased. These may constitute a problem during storage in blood banks.
Keywords : Ascorbic acids, Blood storage, biochemical changes, lipid peroxidation, malondialdehyde.

Introduction

Lipid peroxidation is an oxidative damage of membrane biomolecules especially lipids. It is mediated by oxygen free radicals and reactive oxygen species. Free radicals are atoms or molecular species containing one or more unpaired electrons. They are generally highly reactive species and either to lose an electron, acting as oxidizing agents or to gain an electron, acting as reducing agents [1]. The most important radicals which may be involved in disease processes are species which may be derived from molecular oxygen and certain oxides of nitrogen especially nitric oxide. In addition to lipids, peroxidation affects other biomolecules like proteins, carbohydrates and nucleic acids. Substantial level of peroxidation of membrane biomolecules has the potential of oxidatively damaging cells and tissues of both animal and human. Consequently it has been associated with biotoxicity and diseases [2-3]. This can be exacerbated when total antioxidant status (TAS) is reduced [4]

Ascorbic acid (Vitamin C) is a water-soluble vitamin derived from dietary sources particularly fruits and vegetables. It functions as an antioxidant and is part of antioxidant system of biological tissue [5]. It also facilitates iron absorption [6] and immunologic responses [7].

Storage of donor blood for transfusion induces some biochemical changes in the erythrocytes, which are collectively referred to as lesions of storage [8]. These changes constitute burdens for blood transfusion because they affect the biochemical integrity of transfused erythrocytes. Some vital metabolites like 2,3diphosphoglycerate (DPG), adenosine triphosphate (ATP), lactic acid and lipid content of erythrocyte membranes are affected by some biochemical changes [9,10]. Anticoagulant solutions utilized in blood transfusion contain dextrose (two glucose units), which serves asa metabolic fuel for the erythrocytes. As storage progresses, glucose utilization through glycolysis continues to generate ATP which energizes the cation pump of the membrane and 2,3 DPG, an allosteric effectof haemoglobin function. it can therefore be inferred that storage reduces the functional viability of erythrocytes and this may become more severe as the expiry date approaches.

Blood transfusion is the parenteral infusion of whole blood products collected from an apparently healthy donor into a needy recipient. Storage of donor blood is critical in blood transfusion practice. The inclusion of dextrose has proved very useful in storage of whole blood because erythrocytes utilize it to generate chemical energy necessary for viability. However, it is likely that glucose metabolism may generate increased oxygen free radicals and reactive oxygen species. It has been reported that glucose is a potent source of these reactive species because it can auto-cyclize to produce reactive species[11]. Blood donors are not usually screened for total antioxidant status before blood collection. It is expected that storage will deplete anti-oxidant level of donor blood. At present determination of lipid peroxidation is not included in the cross matching procedures to determine "in vitro" suitability of donor blood for the would-be recipients. In view of the document biotoxic effects of oxygen free radicals/reactive species and possibility of its increased level during storage, it is necessary to determine the level of lipid peroxidation marker malondialdehyde (MDA) and ascorbic acid, a free radical antioxidant during storage of donor blood. This may furnish data about possible adverse effect of transfused blood which is not currently taken into account in compatibility testing.

Materials and Methods:

Study participants

Study participants were blood donors recruited at the University of Nigeria Teaching Hospital (UNTH)Enugu from August 2015 to June 2016. All participants were blood donors that met all the blood donor criteria for donation as required by blood donation and Transfusion guidelines. **Ethical approval and consent to participate**

Consent was sought from all participants before inclusion into the study. The ethics committee and review board of University of Nigeria Teaching Hospital ItukuOzalla Enugu Nigeria.

Sample Collection

Twenty different blood samples were collected from participants into Citrate Phosphate Destrose Adenine (CPDA) anticoagulant. The samples were distributed equally into plastic storage bottles and stored in the blood bank of UNTH at 4– 6^{o}c. The determinations were performed on the first day of collection and second, fourth and sixth weeks of storage.

Determination of MDA concentration

The concentration of MDA was determined by the Thiobarbituric Acid Reading substances Method of Albro et al [12]

Principle

Malondiadeyde(MDA) reacts with thiobarbituric(TBC) acid to form a complexMDA-TAB2 that is measured at 532nm.

Determination of Ascorbic acid concentratiom

Ascorbic acid concentration was determined by the method of Roe and Kenther [13].This uses coupling reaction of dehydroascorbic acid with 2,4dinitrophenylhydrazine for the determination of total ascorbic acid.

Principle:

Ascorbic acid is oxidized to its dehydrate form and then coupled to 2,4dinitrophenylhydrazine to produce a red color which can be measured photometrically.

In this method, *l*- ascorbic acid was oxidized to dehydroascorbic acid by the addition of Norrit to the filtrate. It was incubated for three hours at 37oC with 2,4dinitrophenylhydrazine and thiourea. Colour was developed by the addition of eighty-five percent of sulphuric acid and the mixture read photometrically against a blank.

Statistical analysis

Data was analyzed using statistical package for social sciences (IBM SPSS).Comparisons of the different storage groups was done with analysis of variance (AVOVA) with Duncan Multiple range test were applicable.

Results

The mean concentration of MDA showed a progressive increase as storage progressed. Statistical comparism of the MDA on the first day of collection with values obtained on second, fourth and sixth weeks showed significant differences ($P<0.05$). The highest concentration was obtained in the sample stored for six weeks while the lowest was on the first day of collection.

In the case of ascorbic acid, the result showed a steady decrease. There was no significant difference ($P>0.05$) between values obtained on the first day of collection and second week of storage. However, significant differences ($P<0.05$) were obtained when values of first day of collection was compared with result of fourth and sixth week of storage. The highest concentration was obtained in the first day of collection while the lowest was recorded on the sixth week of collection (table 1). Comparison based on blood groups showed no significant differences ($P>0.05$).

Table 1: The mean values of MDA and Ascorbic acid concentration

Days of collection	**MDA(µg/ml)**	**Ascorbic acid(mg/100ml)**
1st Day of Collection	0.56	1.27
Two weeks of Storage	0.86±0.17	1.16±0.39
Four weeks of Storage	1.81±0.29	0.91±0.1
Six weeks of Storage	2.27±0.14	0.76±0.13

Values are represented as mean±standard deviation. MDA-malondialdehyde

Discussion

Blood transfusion as a supportive and replacement therapy is very critical in medical and surgical management of disease. Biochemical changes during storage of donor blood have continuously constituted a hindrance in ensuring wholesome blood transfusion. Peroxidation of biological membranes by oxygen free radicals and reactive species has been implicated in pathogenesis and pathophysiology of some disease [14-15]The goal of blood preservation is to provide viable and functional components for patients that need blood transfusion. The results obtained in this study suggest that peroxidation of erythrocyte membrane may be one of the lesions of storage, as well as one of the adverse effects of blood transfusion because significant peroxidation may interfere with survival of transfused red cells. The findings of this work is in agreement with that of Devi et al [16] who recorded increased MDA concentration in donor blood stored in (CPDA) plastic bags. The increased lipid peroxidation can lead to oxidative destruction of erythrocyte membrane. When such donor blood is transfused, it may be prematurely destroyed and this may be an additional metabolic burden for the recipient.

The loss in functional integrity of red cells has been correlated with lesion of storage. These changes include a decrease in pH, a build-up of lactic acid, a decrease in both glucose concentration and ATP levels, and loss of red cell function expressed as a shift to the left of the haemoglobin-oxygen dissociation curve or an increase in haemoglobin-oxygen affinity.

The anticoagulant solution utilized in this study contains dextrose, which consists of two units of glucose. It is possible that progressive peroxidation obtained in this study may have been influenced by glucose; oxygen free radicals generated by glucose metabolism have been reported to induce peroxidation of cultured human kidney proximal tubules cells [17]. It has been reported that increased oxygen free radical contributes to oxidant injury and other complications of diabetes mellitus [18]. In a study of the effect of glucose as exogenous substrates on free radical defense mechanism of isolated heart of rats during ischaemic reperfusion, decreases in activity of glutathione reductase, superoxide dismutase (S0D) and catalase were reported [19]. In particularly, the role of S0D in the defense of bacteria exposed to toxicants that generate reactive oxygen species (ROS) has been evaluated[20]. It has been stated that superoxide radical is an important factor in high-dietary carbohydrate lesion [21]. Metabolic utilization of glucose involves redox reactions and bioneroetic election transfer and these predispose it to generate reactive species.

The ascorbic acid concentration declined progressively throughout the period of the study. Although as a dietary substance, it is expected to decline during storage, the pattern suggested that after the second week, the concentration may not be consistent with its antioxidant function. It is also possible for the reduction to be due to increased activity of oxygen free radicals. The quality of the antioxidant is essential to counteract the deleterious effect of reactive species [22]. If blood with low antioxidant status is transfused, it may not be sufficiently beneficial to the recipient. Increased lipid peroxidation of stored donor blood has been suggested [23]. In contemporary management of cardiac surgery patients, production of oxygen-free radicals is utilized as a biocompatibility marker [24]. Although other antioxidants like Vitamin E, Carotene and antioxidant enzymes such as SOD, catalase and peroxidase were not investigated, ascorbic acid is still a quality factor in total antioxidant status while malondialdehyde is a marker of oxidative damage [25].

Considering the role of blood transfusion during surgery and other disease condition and the biochemical changes attendant on storage of donor blood, it is necessary to focus more attention on the issue of depletion in antioxidant and lipid peroxidation level. Currently, determination of lipid peroxidation and total antioxidant status are not included in compatibility tests. In view of the result of this study, it appears that lipid peroxidation may be an additional adverse effect in blood storage and transfusion.

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