Chapter One

Introduction and Literature Review

1.1 Introduction:-

Platelet transfusion is often considered a life-saving measure, being essential for the prevention and treatment of bleeding in patients who have quantitative and/or functional platelet disorders. (Fabris e*t al*. 2000). Nowadays, in many western countries, the demand for platelet concentrates (PCs) is obviously growing, almost up to 80% increase, compared to a decline in the use of packed red blood cells. (Verma and A garwal 2009).

Intensive therapies producing severe and sustained thrombocytopenia are used routinely in patients with hematologic malignancies and are being applied to many patients with solid tumors as well. Advances in platelet collection, storage, and transfusion have decreased the morbidity of such therapies, and death from hemorrhage is now an unusual occurrence, despite the larger number of patients being treated aggressively. (Schiffer *et al*. 2001).

In the 1950s/1960s, platelet transfusions were given as freshly drawn whole blood or platelet-rich plasma. Circulatory overload quickly developed as a major complication of this method of administrating platelets. Since the 1970s, platelets are prepared from whole blood as concentrates in which the volume per unit is near 50 ml in contrast to the 250-to 300-ml volume of platelet-rich plasma units. Today platelets are prepared as concentrates from whole blood and increasingly by apheresis. Platelets still remain as the primary means of treating thrombocytopenia, even though therapeutic responsiveness varies according to patient conditions and undefined consequences of platelet storage conditions. (Harmening, 2005).

Platelets play an important role in the haemostatic process by sealing damaged blood vessels, forming a platelet plug and preventing the blood loss. In transfusion medicine, platelet concentrates were originally used for treatment and prevention of hemorrhage due to severe thrombocytopenia, which is often caused by medullary aplasia, acute leukemia or significant blood loss during long-lasting surgery. However platelets contain high quantities of key growth factors and other bioactive molecules and the expansion of platelet-derived growth factor (PDGF) through applications of platelet-rich plasma (PRP) or platelet gels is thought to stimulate angiogenesis and promote more rapid tissue repair. Therefore Platelet preparations have been used for a wide variety of clinical applications such as oral and maxillofacial surgery, plastic surgery, ophthalmology, orthopedics, treatment of chronic wounds, sports-related injuries, tissue engineering and cellular therapy. (Jameson, 2007; Dohan Ehrenfest *et al.* 2009; Amable *et al.* 2013)

Platelets for transfusion can be prepared either by separation of units of platelet concentrates (PCs) from whole blood, which are pooled before administration, or by aphaeresis from single donors. Comparative studies have shown that the post transfusion increments, hemostatic benefit, and side effects are similar with either product. Thus, in routine circumstances, they can be used interchangeably. In most centers, pooled PCs are less costly. Single-donor platelets from selected donors are preferred when histocompatible platelet transfusions are needed. Both preparations can be stored for up to 5 days after collection at 20°C to 24°C with good maintenance of platelet viability. (Schiffer *et al.* 2001) .

The working definition of PC is a concentration of 1,000,000 platelets/ µl of platelet preparation. In other words, the concentration of platelets should be developed to have a 3 to 5 fold increase over the baseline. (Marx, 2001; Weibrich *et al.* 2004).

In fact the natural variations in platelet concentrations among individuals as well as the daily variation in platelet parameters observed within individuals can further affect the consistency, efficacy and clinical outcomes of the final product. (Wiens *et al.* 2007). In addition, the final platelet concentration of any PRP product is based on the initial volume of the whole blood taken, the platelet recovery efficiency of the technique used, and the final volume of plasma used to suspend the concentrated platelets and changing any of the aforementioned variables will proportionally change the final platelet concentration. (Arnoczky *et al.* 2011).

The quality of PCs is affected by the preparation method of the PCs, the plastic material of the storage bag, the storage conditions including duration of storage, the storage temperature, type of anticoagulant used, the concentration of PCs in the bag and the agitation.(Rivera *et al.* 2004; Tynngard 2009).

In the routine platelet-production laboratory, the quality-control assessments made on platelet concentrates generally include only those measurements required by various standards bodies: platelet concentrate volume, platelet count, pH of the unit, and residual leukocyte count if claims of leukoreduction are made.(Guide to the preparation, use, and quality assurance of blood components. 2007).In addition, immediately before distribution to hospitals a visual inspection is made that often includes an assessment of platelet swirl, an at best semi quantitative reflection of the degree of discoid shape of the platelets within the bag.(Mathai *et al.* 2006).

This study is conducted to assess the quality of platelet concentrates prepared from whole blood using the platelet – rich plasma method. The preparation and storage conditions are checked by measurement of platelet concentrates quality parameters in term of volume, cell count, swilling scores and pH. This will help to evaluate the viability and functional properties of stored platelets so as to ensure efficacy and maximum benefit to the patient.

1.2 Literature review:-

1.2.1 Platelets:-

1.2.1.1 Platelet production:-

Platelets are produced in the bone marrow by fragmentation of the cytoplasm of megakaryocytes, one of the largest cells in the body. The precursor of the megakaryocyte-the megakaryoblast-arises by a process of differentiation from the haemopoietic stem cell .The megakaryocyte matures by endomitotic synchronous replication (i.e. DNA replication in the absence of nuclear or cytoplasmic division) enlarging the cytoplasmic volume as the number of nuclear lobes increase in multiples of two. Very early on invaginations of plasma membrane are seen, called the demarcation membrane, which evolves through the development of the megakaryocyte into a highly branched network. At a variable stage in development, most commonly at the eight nucleus stage, the cytoplasm becomes granular. Mature megakaryocytes are extremely large, with an eccentric placed single lobulated nucleus and low nuclear to cytoplasmic ratio . Platelets form by fragmentation of megakaryocyte cytoplasm, approximately each megakaryocyte giving rise to 1000-5000 platelets. Thrombopoietin is the major regulator of platelet production and is constitutively produced by the liver and kidneys.(Hoffbrand *et al*. 2006).

1.2.1.2 Number and life span:-

The normal range for the platelet count in peripheral blood is about 150–450 × 10^9/l ; slightly lower values are seen during the first 3 months of life. Small cyclical variations in the platelet count may be seen in some individuals of both sexes, with a periodicity of 21–35 days; in premenopausal women the fall usually occurs during the 2 weeks preceding menstruation. The platelet counts of women are slightly higher than those of men.(Stevens and Alexander 1977 ; Bain, 1985). There are also slight racial variations in the normal platelet count. For example,

values lower than those quoted above have been reported in Australians of Mediterranean descent. In addition, Nigerians have lower platelet counts than Caucasians, as have Africans and West Indians living in the UK. (Bain and Seed 1986). The life span of normal platelets is 8–10 days.(Porwit et al. 2011).

1.2.1.3 Platelet structure:-

Platelets are extremely small and discoid, 3.0 x 0.5 μm in diameter, with a mean volume 7-11 fL. The glycoproteins of the surface coat are particularly important in the platelet reactions of adhesion and aggregation which are the initial events leading to platelet plug formation during haemostasis. Adhesion to collagen is facilitated by glycoprotein la (GPla). Glycoproteins Ib (defective in Bernard-Soulier syndrome) and IIb/IIIa (defective in thrombasthenia) are important in the attachment of platelets to von Willebrand factor (VWF) and hence to vascular subendothelium where metabolic interactions occur. The binding site for IIb/IIIa is also the receptor for fibrinogen which is important in platelet-platelet aggregation.

The plasma membrane invaginates into the platelet interior to form an open membrane (canalicular) system which provides a large reactive surface to which the plasma coagulation proteins may be selectively absorbed. The membrane phospholipids(previously known as platelet factor 3) are of particular importance in the conversion of coagulation factor X to Xa and prothrombin (factor II) to thrombin (factor IIa) .The platelet contains three types of storage granules: dense, *a* and lysosomes. The more frequent specific *a* granules contain a heparin antagonist (PF4), platelet-derived growth factor (PDGF), β-thromboglobulin, fibrinogen, VWF and other clotting factors. Dense granules are less common and contain adenosine diphosphate (ADP), adenosine triphosphate (ATP), 5-hydroxytryptamine (5-HT) and calcium. Lysosomes contain hydrolytic enzymes and peroxisomes contain catalase. Platelets are also rich in signalling and cytoskeletal proteins which

support the rapid switch from quiescent to activation that follows vessel damage. (Hoffbrand *et al*. 2006) .

1.2.1.4 Platelet function in the haemostatic process :-

The main steps in platelet functions are adhesion, activation with shape change, and aggregation. When the vessel wall is damaged, the subendothelial structures, including basement membrane, collagen and microfibrils, are exposed. Surface-bound vWF binds to GpIb on circulating platelets resulting in an initial monolayer of adhering platelets. Binding via GpIb initiates activation of the platelet via a G-protein mechanism. Once activated, platelets immediately change shape from a disc to a tiny sphere with numerous projecting pseudopods. After adhesion of a single layer of platelets to the exposed subendothelium, platelets stick to one another to form aggregates. Fibrinogen, fibronectin and the glycoprotein Ib- IX and IIb/IIIa complexes are essential at this stage to increase the cell to cell contact and facilitate aggregation. Certain substances (agonists) react with specific platelet membrane receptors to promote platelet aggregation and further activation. The agonists include : exposed collagen fibers, ADP, thrombin, adrenaline, serotonin and certain arachidonic acid metabolites including TXA2. In areas of non-linear blood flow such as may occur at the site of an injury, locally damaged red cells release ADP which further activates platelets.(George, 2000).

1.2.2 Platelet Transfusion:-

1.2.2.1 History of platelet transfusion

Unlike red cells, the history of platelet transfusions is short and has only developed over the past 40 years. However, it was in 1910 that Duke first noted that platelets from whole blood reduce the bleeding time. (Duke, 1910). This was the first evidence of a potential role for platelet transfusion therapy. In the 1950s platelets were collected and transfused, but only for diagnostic purposes. In the 1960s plastic

blood bags became available which enabled platelets to be separated from whole blood collections by centrifugation. In the 1970s it was discovered that platelet function was best preserved if the platelets were stored at room temperature with agitation. This resulted in the introduction of prophylactic platelet transfusions for patients with leukaemia.New methods for platelet transfusion were introduced in the 1980s using cytapheresis techniques and this was further refined in the 1990s with methods of collecting leucocyte reduced platelet products. The success of platelet transfusion therapy has been critical for developments in many areas of modern medical and surgical management.(Erber *et al*. 2001).

1.2.2.2 Indications:-

Platelet transfusions may be given to patients with thrombocytopenia, dysfunctional platelet disorders (congenital, metabolic, or medication-induced), active platelet-related bleeding, or at serious risk of bleeding (ie, prophylactic use). Patients with the following medical conditions may require platelet transfusion: leukemia, myelodysplasia, aplastic anemia, solid tumors, congenital or acquired platelet dysfunction, and central nervous system trauma. Patients undergoing extracorporeal membrane oxygenation or cardiopulmonary bypass may also need platelet transfusion, and platelets may be indicated in massive transfusion protocols. Thrombocytopenia is unlikely to be the cause of bleeding in patients with platelet counts of at least 50,000/μL. Higher transfusion thresholds may be appropriate for patients with platelet dysfunction. For the clinically stable patient with an intact vascular system and normal platelet function, prophylactic platelet transfusions may be appropriate at <5000 to 10,000/μL. Prophylactic platelet transfusion may not be of therapeutic benefit when thrombocytopenia is related to destruction of circulating platelets secondary to autoimmune disorders [eg, immune thrombocytopenic purpura (ITP)]; however, transfusion may be indicated for active

bleeding in these patients. Platelets Leukocytes Reduced or Apheresis Platelets Leukocytes Reduced are indicated to decrease the frequency of recurrent febrile, nonhemolytic transfusion reaction, HLA alloimmunization, and transfusion-transmitted CMV infection. (AABB, the American Red Cross, America's Blood Centers, and the Armed Services Blood Program 2013)

1.2.3 Platelet Products:-

Platelets for transfusion can be prepared either by separation of units of platelet concentrates (PCs) from whole blood, which are pooled before administration, or by apheresis from single donors. (Schiffer *et al*. 2001).Each unit of platelets (concentrate) must contain at least $5.5x10^{10}$ platelets.(American Association of Blood Banks, Bethesda and MD 2002) and should increase the platelet count 5000 to 10,000/μL in the typical 70-kg human. Pools of 4 to 6 units, then, will contain roughly $3x10^{11}$ platelets and should give a platelet increment of 20,000 to 60,000//μL. (Harmening, 2005).

1.2.3.1 Platelet concentrates from Whole Blood:-

Often referred to as random donor platelets, PCs are prepared by centrifugation of standard units of whole blood .There are two methods for doing this: (1) the platelet-rich plasma (PRP) method, and 2) the buffy coat (BC) method. (Murphy , Heaton and Rebulla 1996). The PRP method is used in the United States, whereas the BC method is in common use in Europe. In the PRP method, an initial low G force (soft) spin produces PRP, which is separated from the red cells. The PRP is then centrifuged at a higher G force (hard) spin, and most of the platelet-poor plasma is removed.(Slichter and Harker 1976; Kahn *et al*. 1976; Reiss and Katz 1976 ; Slichter and Harker 1976). The residual (PCs) contain approximately 0.5 to 0.75 x 10^{11} platelets/unit or approximately 60% to 75% of the platelets from the original unit of whole blood. Because some blood centers now supply units with

higher numbers of platelets, clinicians should be aware of the average dose provided by their particular center. One drawback to this method is that the resulting PCs also contain 10^8 to10^9 WBCs or approximately 50% or more of the leukocytes from the original unit of whole blood. (Schiffer et al. 2001)

PCs can also be obtained from 40- to 50-mL BCs collected at the red cell/plasma interface after high-speed centrifugation of 450 mL whole-blood donations. (Hogman *et al*. 1997; Pietersz *et al*. 1985; Eriksson *et al*. 1993). After gentle resuspension in a satellite bag, the BC is centrifuged at low speed and the platelets collected in the supernatant. (Pietersz *et al*. 1985). Alternatively, four to six BCs are pooled, diluted in plasma, and centrifuged at low speed to suspend the platelets in the supernatant, which is then transferred into a large-volume storage bag. Plasma can be replaced with a crystalloid platelet additive solution, thus reducing the amount of plasma that might be infused to plasma-incompatible recipients. (Bertolini *et al*. 1992 ; Eriksson *et al*. 1993).The BC-PCs must be used within 6 hours of preparation if the bags have been entered during pooling. Storage can be extended to 5 days if the whole procedure is performed in closed systems. A number of studies indicate that BC-PCs produce comparable in vivo platelet survival and contain similar numbers of less-activated platelets and fewer white cells compared with PCs prepared with the PRP method. (Keegan *et al*. 1992 ;Heaton *et al*. 1997; van Rhenen *et al*. 1998).

1.2.3.2 Single-Donor Platelets Produced by Apheresis:-

Although the Food and Drug Administration term for this component is “platelets, apheresis,” the component is usually called single-donor platelets. Donors usually underg two venipunctures. Blood pumped from one vein passes through a blood-cell separator centrifugation system with removal of the platelets

or other cellular components and return of the plasma and RBCs to the donor's other arm.

Platelet pheresis usually requires approximately 11/2 to 2hours and involves processing 4,000 to 5,000 mL of the donor's blood. (Katz *et al*. 1981; Buchholz *et al*. 1983; Hogge and Schiffer 1983; Schoendorfer *et al*. 1983; Simon *et al*. 1991; Kuriyan and Opalka 1995). After repeated cycles the platelets are concentrated in storage containers .Apheresis components contain at least four to six times as many platelets as a unit of platelets obtained from whole blood .This translates into a product with 3.0 to 4.0 $x10^{11}$ platelets, which provides one transfusion dose. AABB Standards now require that 90 percent of the tested units have a minimum of 3.0 x 10^{11} platelets. (American Association of Blood Banks, Bethesda and MD 2002). Each apheresis product has a volume of approximately 200 mL and contains few red cells, so that red cell cross matching is not necessary. The WBC content varies, depending on the instrument and technique used for collection, but most plateletpheresis products now contain less than 5 X 10^6 leukocytes and can be considered to be leukocyte reduced.(Schiffer *et al*. 2001).

1.2.4 Platelet Storage:-

Platelet concentrates prepared from whole blood and apheresis cmponents are routinely stored at 20˚to 24˚C with continuous agitation for up to 5 days on being prepared using a system classified as being closed, bags, and/or cell separators with apheresis collections.FDA standards define the expiration time as midnight of day 5.(Harmening, 2005) .

1.2.4.1 History of Platelet Storage; Rationale for Current Conditions:-

The conditions utilized to store platelets have evolved since the 1960s as key parameters that influence the retention of platelet properties have been identified. Initially, platelets were stored in the cold at 1˚ to 6 ˚C, based on the

successful storage of RBCs, as whole or separated RBC components at this temperature range. A key study report in 1969 by Murphy and Gardner showed that cold storage at 1˚ to 6 ˚ C resulted in a marked reduction in platelet in-vivo viability, manifested as a reduction in in-vivo life span, after only 18 hours of storage. (Murphy and Gardner 1969) This study also identified for the first time that 20˚ to 24˚ C (room temperature) should be the preferred range, based on viability results. The reduction in viability at 1˚ to 6 ˚C was associated with conversion of the normal discoid shape to a form that is irreversibly spherical. This structural change is considered to be the factor responsible for the deleterious effects of cold storage. When stored even for several hours at 4 ˚ C, platelets do not return to their disc shape upon warming. This loss of shape is probably a result of microtubule disassembly.(Harmening, 2005) .

One factor identified as necessary was the need to agitate platelet components during storage, although initially the rationale for agitation was not understood. (Slichter and Harker 1976 ; Murphy 1985). Subsequently, agitation has shown to facilitate oxygen transfer into the platelet bag and oxygen consumption by the platelets. The positive role for oxygen has been associated with the maintenance of platelet component pH.(Murphy and Gardner 1975). Maintaining pH was determined to be a key parameter for retaining platelet viability, in-vivo when platelets were stored at 20˚ to 24 ˚ C. A pH of 6.0 was initially the standard for maintaining satisfactory viability. The standard was subsequently changed to 6.2 with the availability of additional data. As pH is reduced from 6.8 to 6.2/6.0, the platelets progressively changed shape from discs to spheres. Much of this change was irreversible.

The reduction in pH, in the presence of agitation, was shown to be due to a decrease in plasma oxygen levels that was associated with the channeling of platelet metabolism from the aerobic respiratory pathway to the anaerobic

glycolytic pathway. With glycolysis, glucose is converted to lactic acid, which depletes the plasma bicarbonate and hence the plasma constituent that allows for the maintenance of pH (Murphy and Gardner 1975). The gas transport proprieties of acontainer is known to reflect the container material, the gas permeability of the wall of the plastic container, the surface area of the container available for gas exchange, and the thickness of the container. (Harmening, 2005) .

1.2.4.2 Measurement of Viability and Functional Prosperities of Stored platelets:-

Viability indicates the capacity of platelets to circulate after infusion without premature removal or destruction. Platelets have a life span of 8 to 10 days after release from megakaryocytes. Storage causes a reduction in this parameter, even when pH is maintained. Platelet viability of stored platelets is determined by measuring pretransfusion and post transfusion platelet counts.(1 hour and/or 24 hours) and expressing the difference based on the number of platelets transfused (corrected count increment) or by determining the disappearance rate of infused radio labeled platelets to normal individuals whose donation provided the platelets.

The observation of the swirling phenomenon caused by discoid platelets, when platelet suspensions, without sampling, are placed in front of a light source, has been used to obtain a qualitative evaluation of the retention of platelet viability properties in stored units(Bertolini and Murphy 1996).The extent of shape change and the hypotonic shock response in in-vitro tests appear to provide some indication about the retention of platelet viability properties (Holme *et al*. 1998). Function is defined as the ability of viable platelets to respond to vascular damage in promoting hemostasis, clinical assessment of hemostasis is being increasingly used. The template bleeding time test also has been used to assess the functional integrity of transfused platelets, but in recent years there have been questions about

the specificity of this procedure. The maintenance of pH during storage at 20˚ to 24 ˚C has been associated with the retention of post transfusion platelet viability and has been the key issue that has been addressed to improve conditions for storage at 20˚ to 24 ˚C (Harmening, 2005) .

1.2.4.3 Platelet Storage and Bacterial Contamination:-

The major concern associated with storage of platelets at 20˚ to 24 ˚C is the potential for bacterial growth, if the prepared platelets contain bacteria because of unremoved contamination at the phlebotomy site or the donor has unrecognized bacterial infection (Brecher and Hay 2003).Room temperature storage and the presence of oxygen provide a good environment for bacterial proliferation. Many studies have shown that approximately 1 to 3 of every 1000 prepared platelet units contain bacteria. Although the level of patient sepsis is much lower, particularly troublesome is the fact that some septic episodes have led to patient deaths.

Two systems that involve the culturing of platelet samples for approximately 24 to 30 hours have been documented to provide good sensitivity and specificity. (Macauley *et al*. 2003). There are also less sensitive methods that need to be used prior to transfusion. One method is gram staining. Another procedure involves the use of clinical chemistry dipsticks that measure pH and glucose levels, as substantial bacterial growth can be associated with low pH and glucose levels.(Wagner and Robinette 1996).

1.2.4.4 Storage with Additive Solutions:-

Currently in Europe, additive (synthetic) solutions are being used to replace a large portion of the plasma in platelet suspensions prepared from whole blood by the buffy coat method. Residual plasma is about 20 percent to 35 percent. (De Wildt-Eggen and Gulliksson 2003). Platelet additive solutions in use/being developed contain varying quantities of glucose, citrate, phosphate, potassium,

magnesium, and acetate. Acetate is a primary constituent as it serves as a substrate for aerobic respiration (mitochondrial metabolism) while also providing away to maintain pH levels as it reacts with hydrogen ions when it is first utilized (Harmening, 2005).

1.2.5 Dosage and Administration:-

Compatibility testing is not necessary in routine platelet transfusion. Except in unusual circumstances, the donor plasma should be ABO compatible with the recipient's red cells when this component is to be transfused to infants, or when large volumes are to be transfused. The number of platelet units to be administered depends on the clinical situation of each patient. One unit of Platelets would be expected to increase the platelet count of a 70-kg adult by 5000 to 10,000/μL and increase the count of an 18-kg child by 20,000/μL. The therapeutic adult dose is 1 unit of Apheresis Platelets or 4 to 6 units of whole blood-derived platelets, either of which usually contain $\geq 3.0 \times 10^{11}$ platelets. For prophylaxis, this dose may need to be repeated in 1 to 3 days because of the short lifespan of transfused platelets (3 to 4 days).

Platelet components must be examined for abnormal appearance before administration. Units with excessive aggregates should not be administered. Transfusion may proceed as quickly as tolerated, but must take less than 4 hours.

The corrected count increment (CCI) is a calculated measure of patient response to platelet transfusion that adjusts for the number of platelets infused and the size of the recipient, based upon body surface area (BSA) CCI = (postcount – precount) × BSA / platelets transfused where postcount and precount are platelet counts (/μL) after and before transfusion, respectively; BSA is the patient body surface area (meter2); and platelets transfused is the number of administered platelets ($\times 10^{11}$). The CCI is usually determined 10 to 60 minutes after transfusion.

In the clinically stable patient, the CCI is typically greater than 7500 at 10 minutes to 1 hour after transfusion and remains above 4500 at 24 hours. Both immune and nonimmune mechanisms may contribute to reduced platelet recovery and survival (AABB, the American Red Cross, America's Blood Centers, and the Armed Services Blood Program 2013).

1.2.5.1 Selection of Platelets:-

1.2.5.1.1 ABO Matching:-

Because ABO antigens are present on the platelet surface, recovery of group A platelets transfused into group O patients is somewhat decreased (Aster, 1965) but this effect is not usually clinically significant. Transfusion of ABO-incompatible plasma present in platelet components may also result in a blunted posttransfusion platelet count increment (Heal *et al*. 1993) Hemolysis occurs rarely in this setting but it frequently causes a positive direct antiglobulin test (DAT), which may increase costs and charges to the patient for serologic investigation. Moreover, a retrospective analysis suggested that survival after marrow transplantation was significantly reduced in patients who received substantial amounts of ABO-incompatible plasma from platelet transfusion, (Benjamin and Antin 1999) and it has been suggested that infusion of soluble A and B antigen in platelet or plasma components may have a similar adverse effect mediated by immune complex function (Heal and Blumberg 1999).

1.2.5.1.2 Matching for Rh:-

The D antigen is not detectable on platelets, and post transfusion survival of platelets from Rh-positive donors is normal in recipients with anti-D. However, platelet components contain small numbers of red cells so Rh-negative individuals may become alloimmunized by platelet components from Rh-positive donors. For

immunocompetent normal Rh-negative females of childbearing potential, it is especially desirable to avoid administration of platelets from Rh-positive donors; however, if this is unavoidable, Rh Immune Globulin (RhIG) should be administered. (Mark and Brecher 2005).

1.2.6 Refractoriness to Platelet Transfusion:-

Platelet refractoriness, defined as a poor increment following a dose of platelets, can result from either immune or nonimmune mechanisms (McFarland 2003; Kickler, Herman and eds 1999). The antibodies that cause immune refractoriness may have either allo- or auto reactivity, with alloantibodies most commonly directed against Class I HLA antigens. Autoantibodies occur in immune thrombocytopenic purpura (ITP). Nonimmune causes of the refractory state include infection, splenomegaly, drugs (particularly amphotericin B), and accelerated platelet consumption (Mark and Brecher 2005).

1.2.7 Quality assessment of platelet concentrates:-

Quality assessment of platelet concentrates is an important step to evaluate *ex-vivo* functional viability of platelet concentrates and post transfusion recovery and survival in recipients. Various parameters are used for routine *ex-vivo* quality assessment of platelet concentrates such as swirling, volume, platelet count, WBC count and pH. Although other parameters are also used such as, measurements of ATP, membrane glycoprotein levels (P-selectin, GP Ib, GP IIb-IIIa) etc., these tests are cumbersome, not well standardized and difficult to perform on every PC unit in a routine setting. The *in vivo* viability of a transfused platelet product is determined by the percentage of the transfused platelets recovered in the recipient's circulation immediately after transfusion (% recovery) and by the life span in circulation of these recovered platelets (survival) (Holme, 1998).

1.2.7.1 Swirling:-

Discoid platelets exposed to a light source reflect light and thus produces the "swirling" phenomenon. Swirling is routinely used to evaluate the quality of platelet concentrates (PC). Swirling determinations are performed by examining a PC against a light source while gently rotating the container or gently squeezing the PC. The presence of swirling indicate a pH value within the adequate range (Bertolini and Murphy 1996).

1.2.7.2 Volume:-

Platelets prepared from whole blood collections or plateletpheresis are stored in donor plasma, which serves as a buffering agent. PCs from RDPs are typically suspended in 40 to 70 ml plasma to maintain pH. The major reason for using this volume range was based on early studies with PCs stored in first generation PVC containers. Because of the insufficient permeability of these containers to oxygen, there was a risk of a drop in the pH in the PCs from anaerobic conditions and elevated lactic acid production. The platelet-suspending volume was, therefore, maximized to increase buffering capacity while maintaining as little volume as possible, to minimize the risk of volume overload of the recipient's circulatory system(Holme *et al*. 1994).

1.2.7.3 Platelet count:-

One unit of Platelets derived from a whole blood collection usually contains $>5.5 \times 10^{10}$ platelets suspended in 40 to 70 mL of plasma. Platelets may be provided either singly or as a pool. One unit of Apheresis Platelets usually contains $\geq 3.0 \times 10^{11}$ platelets and is the therapeutic equivalent of 4 to 6 units of Platelets

(AABB, the American Red Cross, America's Blood Centers, and the Armed Services Blood Program 2013).

In fact the natural variations in platelet concentrations among individuals as well as the daily variation in platelet parameters observed within individuals can further affect the consistency, efficacy and clinical outcomes of the final product (Wiens, et al. 2007) . In addition, the final platelet concentration of any PRP product is based on the initial volume of the whole blood taken, the platelet recovery efficiency of the technique used, and the final volume of plasma used to suspend the concentrated platelets and changing any of the aforementioned variables will proportionally change the final platelet concentration (Arnoczky *et al*. 2011).

1.2.7.4 WBC Count:-

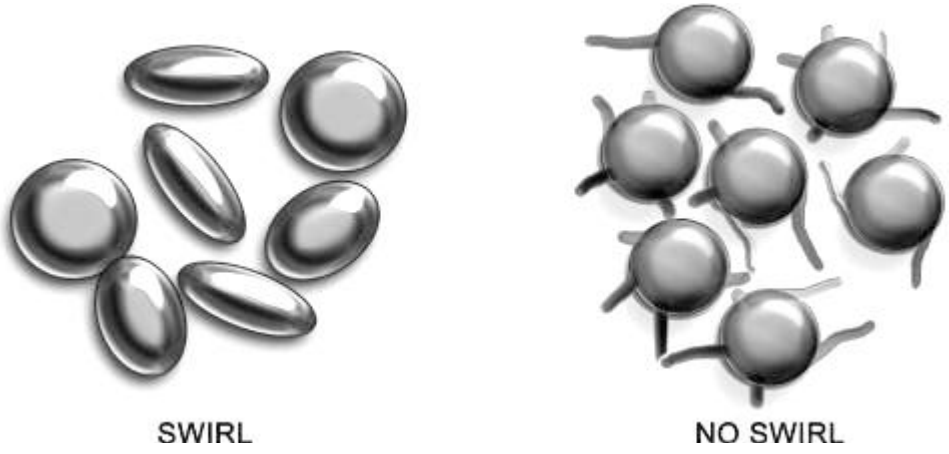
WBCs in PC have a detrimental effect on the storage medium, resulting in a significant drop in pH, increase in glucose consumption, lactic acid production and LDH release during storage. As a result, in the PCs with high concentration of leukocytes, the platelet condition up to 5 days of storage was also significantly affected, as reflected by a high excretion of β-TG, loss of platelet nucleotides, decreased ability to incorporate (Saran, 2003) H-adenosine and poor platelet morphology (Pietersz *et al*. 1988) In addition to these, transfused passenger leukocytes during platelet therapy may be associated with a variety of adverse effects, including alloimmunization to leukocyte antigens, febrile non-hemolytic transfusion reaction (FNHTR), refractoriness to platelet transfusion, severe pulmonary dysfunction, graft versus host disease (GVHD), the transmission of cytomegalovirus (CMV) and immune modulation (Lane, 1994) Platelet concentrates made from individual units of fresh whole blood may contain from 0.5×10^8 to 2.5×10^8 WBC/unit (Merryman and Hornblower 1986). Apheresis platelets that have been harvested using old instruments may contain up to 5×10^9

leukocytes, while apheresis platelets obtained using more recently available instruments contain from 10^6 to 5×10^8 WBCs (Bertnolf and Mintz 1989).

1.2.7.5 pH change:-

The pH decreases during storage depending on the stabilizer in plastic platelet storage bags and storage conditions used. Increased platelet glycolysis resulting in a fall in pH to levels approaching 6.0 in PC stored in plasma is associated with substantial loss of viability (Gulliksson, 2003). The majority of fresh, un-stimulated platelets are discoid with few projections. In the early observations of PCs stored at 20-24° C, a gradual disc-to-sphere transformation was seen during storage.

Some of these changes are reversible with incubation at 37° C in fresh plasma. Qualitatively similar changes occur during PC storage, but in first generation containers major additional variable is pH fall. If pH does not fall to less than 6.8, platelet volume decreases by approximately 10% during three days. However, if pH falls below this level, there is a progressive rise in platelet volume and decrease in density suggesting swelling due to influx of extracellular fluid. The swelling begins at pH of 6.8 and reaches its maximum at a pH of 6.0, at which point platelet volume is increased almost two-fold. At the same time, there is an accelerated rate of disc-to- sphere transformation so that only swollen spheres are seen if pH reaches 5.7 to 5.9. These changes are almost entirely reversible if pH stays above 6.1, but they are not reversible if pH falls below 6.1. These morphological observations correlate well with the results of viability in vivo (Murphy, 1985).



A **B**

Figure 1.1: A, when platelets are held up to a light source and gently agitated, swirling platelet scatter incident light at all angles because platelets change orientation relative to the incident light. B, The swirling phenomenon is not observed in non discoid platelets because of their decreased ability to change orientation and scatter light. Adapted by Maurer-Spurej & Chipperfield, 2007 (112).

1.2.8 Previous Studies:-

In study conducted at Kenyatta National Hospital (KNH), **Richard *et al*. (2014)** reported that a total of 78 platelet concentrates were analyzed. The majority 54, (69.2%) were group O RhD+, Group O RhD- was the least frequent blood group at 1.3%. A total of 77, (98.7%) of all concentrates were RhD positive. Only 51% (40) of all concentrates fulfilled the minimum specification for platelet count of >5.5 x 10^{10}; the mean SD for platelet count was 6.63 ± 4.73 x 10^{10} with a median of 5.58 x 10^{10} and a range of 0.89 - 21.50 x 10^{10}. None of the concentrates fulfilled specification for residual WBC count, (Mean + SD, Median and range of residual WBC count are 545 ± 429, 4.40 and 0.08 – 18.9(106) respectively) whereas 91% (71) and 95% (74) of the concentrates fulfilled the standards for volume and pH respectively.

In another study at a tertiary hospital in Nigeria where platelet concentrates were prepared by PRP method, **Fasola *et al*. (2002)** reported that fifty platelet concentrates were assessed for pH, swirling index, volume, platelet count, WBC count and red cell count. All the concentrate preparations were within acceptable pH value of 7.25. The average volume was 18.52 mls/bag. The average platelet count per concentrate was 41.7+/-39.5 x 10(9)/L. Thirty-five percent (35%) of the platelet concentrates had a value > 55 x 10(9)/L. White blood cell count (WBC) < 12 x 10(9)/L was seen 49% of the platelet concentrates prepared. Forty percent (40%) of the platelet concentrate had a red blood cell count (RBC count) > 12 x 10(9)/L with 30% not having red cell contamination. Swirling test was positive in 72% of the platelet concentrate units**.**

In the study conducted in the departments of Transfusion Medicine of the institute, The Mission Hospital , India , **Singh *et al*. (2009)** assessed PRP, BC and apheresis platelets . A total of 146 platelet concentrates (64 of PRP-PC, 62 of BC-

PC and 20 of apheresis-PC) were enrolled the study. The mean volume of PRP-PC was 62.30±22.68 ml and ranged from 22-135 ml and 75.4% (48/64) of PRP-PC unit fulfilled the quality control criteria of volume. The mean platelet count of PRP-PC was 7.6±2.97 × 10^{10}/unit and ranged from 3.2 –16.2 × 10^{10}/unit and Seventy-eight point two percent (50/64) of PRP-PC had platelet counts >5.5 × 10^{10}/unit. The mean WBC count in PRP-PC was 4.05±0.48 × 10^{7}/unit and ranged from 3.4 -4.77 × 10^{7}/unit and it fulfilled the recommended Q.C. criteria for WBC count (Saran, 2003) . A total of 26 units were analyzed for pH changes. Out of these units, 10 each were PRP-PC and BC-PC and 6 units were apheresis-PC. Their mean pH was 6.7±0.26 (mean±SD) and ranged from 6.5 – 7.0 and no difference was observed among all three types of platelet concentrate so that all the platelet concentrate units had pH well above the recommended norm.

In study conducted in the National Transfusion Center (Khartoum) **Khattab *et al.* (2013)** reported that a total of 100 bags of platelet concentrates that, were prepared from random donor whole blood collected locally in the blood bank (62 bags) and from mobile sessions (38bags), were investigated at day one and 75 bags of them were stored five days and the same investigations repeated at day five. The quality parameter of the 100 bags of platelets concentrates investigated at day one showed volume range between 43-120ml with a mean of 71ml +/-13.9(54% have a volume of 50-70ml) and a platelet count range between 0.4-11.6x10^{10} with a mean of 5.5+/-2.5(50% have a count of > 5.5 x10^{10}).The swirling score ranged between (+1) – (+3) with a mean of +3 (87 % have score +3) and the pH ranged between 6.9-8.2 with a mean of 7.5+/-0.3 (100 % have a pH > 6.2).

1.3 Rationale :-

Numerous steps are involved in platelets concentrates collection, preparation and storage which need to be strictly controlled to ensure the safety of patients and to prevent adverse events. This study is conducted in order to assess the quality of platelets concentrates prepared from random donor whole blood at Elmek Nimer University Hospital Blood Bank in the efforts to provide platelets concentrates that meet the quality requirements specifications.

1.4 Objectives:-

.General objectives :

This study aims to assess the quality parameters of platelet concentrates prepared from random donor whole blood using the platelet-rich plasma method at Elmek Nimer University Hospital Blood Bank.

.Specific objectives:

- To assess the processes used in the collection and preparation of the platelet concentrates.
- To estimate the quality parameters of platelet concentrates by measuring of volume, cells counts, pH and swirling.
- To determine whether these quality parameters met the minimum quality requirement of AABB recommended quality standards.
- To assess the effect of the storage conditions on the quality of platelet concentrates.

Chapter Two

Materials and Methods

2.1 Materials:-

2.1.1 Study Design:-

The study was a descriptive and analytical prospective cross-sectional study conducted in the period from February to April 2015 to assess the quality parameters of platelet concentrates prepared at Elmek Nimer University Hospital Blood Bank.

2.1.2 Study population:-

Platelet concentrates units prepared from the blood collected from the donors who visited Elmek Nimer University Hospital Blood Bank.

2.1.3 Study setting:-

The study was conducted at Elmek Nimer University Hospital Blood Bank, in Shensi town which is located in River Nile State about 172 Km from Khartoum city.

2.1.4 Sampling:-

2.1.4.1Sample size :-

Fifty Platelet concentrates units prepared from whole blood using the platelet-rich plasma method.

2.1.4.2 Inclusion Criteria:-

Platelet concentrates units prepared from blood collected from healthy donors with no recent history of aspirin intake.

2.1.4.3 Exclusion Criteria:-

Donors taking aspirin within the previous 72 hours from donation.

2.1.4.4 Sampling technique:-

Blood samples were obtained from 50 donors, chosen randomly from the donation department at Elmek Nimer University Hospital Blood Bank, Shendi town.

2.1.5 Data collection tool:-

The data was collected using a questionnaire including the donor informations and drug history particularly aspirin.

2.2 Methodology:-

2.2.1 Platelet concentrates collection, preparation and storage:-

2.2.1.1 Platelet collection:-

- Fifty platelet concentrate units freshly collected in triple blood bags containing 63 ml of CPDA-1 anticoagulant, (**JMS Singapore Pte Ltd**), using blood collection monitor (**Hemoscale MODEL AB-20E, JMS-CO-LTD, Japan)** from healthy blood donors who visited the donation department of Elmek Nimer University Hospital Blood Bank.
- A base line platelet count was done for each donor and blood was drown from those with the normal platelet count.

- The unique laboratory identification number, date and time of blood bags collection and ABO/RhD group were recorded for each bag.
- The volume of blood collected in each bag was adjusted to 450 ml by the blood collection monitor and also the time taken for phlebotomy was within 6-8 minutes.
- The bags collected were stored at room temperature, and the temperature was maintained at 22-24 ˚C and the holding time before processing of blood was from 4-6 hours after collection.

2.2.1.2 Platelet concentrates preparation:-

- Platelet concentrates in this study were prepared from whole blood using the platelet-rich plasma method.
- The temperature of refrigerated centrifuge (**ROTANTA 460 R, Hettich, Germany)** was set at 25˚C .
- The whole blood was centrifuged by using a "light" spin (2800 rpm for 3:30 minutes).
- Platelet- rich plasma (PRP) was expressed into the satellite bag intended for platelet storage using plasma expresser (**SEPARATION STAND, ERUFLEX, ACS-201, TURUMO, Japan).** The PRP bags were centrifuged at 25˚C using a "heavy" spin (3200 rpm for 10 minutes).
- The platelet-poor plasma (PPP) was expressed into the second transfer bag without disturbing the platelet button.
- The volume of the platelet concentrates was adjusted to 50 to 70 mL with plasma.
- Following preparation, PCs were left for 1hour without agitation at room temperature for resting highly activated platelets during preparation.

2.2.1.3 Platelet concentrates storage:-

After the I hour at room temperature PCs units were kept on a flatbed platelet agitator **(Agitator TA-1, SELUTEC GmbH, Germany)** then stored with continuous gentle agitation to prevent clumping and facilitate gas exchange at 22-24oC for a total of 5 days.

2.2.2 Quality assessment of platelet concentrates:-

Platelet concentrates quality parameters which include platelet concentrate volume, cell counts, swirling and pH were measured on day one (24 hours post collection) and day five. Approximately 4 ml was drawn from platelet concentrate units under sterile conditions on day one for the purpose of measurement of cell counts and pH.

2.2.2.1 Volume measurement:-

The volumes of PCs were determined by subtracting the weight of empty bag from that of full bag. To convert weight to volume, resultant weight was divided by 1.03 specific gravity of PRP-PC.

2.2.2.2 Cell counts:-

Platelets count, total white blood cells and total red blood cells in the platelet concentrates were measured using the auto hematology analyzer **(BC-3000 Plus, Mindray, China).**

2.2.2.2.1Measurement principles:-

Platelets, red blood cells and white blood cells are counted and sized by the impedance method. This method is based on the measurement of changes in electrical resistance produced by a particle, which in this case is a blood cell, suspended in conductive diluents as it passes through an aperture of known dimensions. An electrode is submerged in the liquid on both sides of the aperture; a transitory change in the resistance between the electrodes is produced. This

change produces a measureable electrical pulse. The number of pulses generated indicates the number of particles that passed through the aperture. The amplitude of each pulse is proportional to the volume of each particle. Each pulse is amplified and compared to the internal reference voltage channels, which only accepts the pulses of certain amplitude. If the pulse generated is above the RBC/PLT lower threshold, it is counted as an RBC/PLT and if the pulse generated is above the WBC threshold, it is counted as a WBC.

The platelet count, white blood cells count and red blood cells count per platelet concentrates units are calculated using the following formulas:-

Platelet count per unit =N $x10^6$ x platelet concentrate unit volume.

WBCs count per unit =N $x10^6$ x platelet concentrate unit volume.

RBCs count per unit =N $x10^9$ x platelet concentrate unit volume.

N (The count obtained from the automated hematology analyzer).

2.2.2.3 Swirling:-

2.2.2.3.1 Principle of the method:-

The swirling effect correlates with the in vitro transformation of platelets from disk to spiny spheres. When platelets are held up to a light source and gently agitated, discoid platelets will scatter incident light at all angels due to change in orientation relative to the incident light. Platelets, which have undergone transformation to spheres, have immediately very little ability to change orientation. The swirling phenomenon is consequently not observed in transformed platelets. Degree of swirling is usually scored from 0 (no swirling) to 3 (maximal swirling).

The swirling was evaluated by examining the units against light and scored as:

- Score 0: Homogen turbid and is not changed with pressure.
- Score 1: Homogen swirling only in some part of the bag and is not clear.
- Score 2: Clear homogenic swirling in all part of the bag.
- Score 3: Very clear homogen swirling in all part of the bag.

2.2.2.4 PH measurement:-

2.2.2.4.1 Principle of the method:-

The principle of the operation is measurement of the potential of a specific ion in solution against a stable reference electrode of constant potential. The method is based on the technique of potentiometry, which is the measurement of electrical potential without a current flowing.

pH of all samples was measured immediately after sampling at a temperature of 22^{o} C using handheld pH meter (**HANNA Instruments HI 96107, pH Tester, Italy**). The electrode of pH meter was placed in PCs and swirled the solution. The pH reading had to be stabilized before the pH result of PCs taken. When the reading was freezed, the pH of PCs was recorded.

2.3 Data Analysis:-

Data was analyzed by the computer program. (SPSS version 16)

2.4 Ethical consideration:-

Ethical approval from the university, hospital administration, and verbal consent from all donors was obtained.

Chapter Three

Results

3.1 Blood groups:-

A total of 50 platelet concentrates were analyzed. The majority 70% , (35) were group O RhD+, 16%, (8) were group A RhD+, 8%,(4) were group B RhD+ and 6%, (3) were group O RhD- . A total of 47, (94%) of all concentrates were RhD+, as shown in table (3-1).

3.2 Quality parameters of platelet concentrates at day one:-

The quality parameters of the 50 platelet concentrate units were investigated at day one and recorded a volume range from 50-93 ml with a mean of 72.06±10.42. We found 38 %,(19) of the platelet concentrate had a volume of 50-70 ml. Then the mean platelet count was 5.09±1.51 and rang from 2.2-9.9x10^{10} /Unit, 40 %, (20) had a platelet count of >5.5x10^{10}/Unit. The residual white blood cells had a mean of 18.96±16.69 and range from 0.0-83.6x10^{6}/Unit. The red blood cells had a mean of 1.41±0.78 and range from 0.0-3.20x10^{9}/Unit. Swirling score had a mean of 2.94±0.24 and range from 2-3 score. For pH assessment 47 platelet concentrates were tested (3 samples was lost during analysis) and the mean pH was 7.94±0.20 and range from 7.4-8.2 .The findings were shown in table (3-2).

3.3 Characteristic of platelet concentrates with platelet count of >5.5x10^{10}/Unit:-

The platelet concentrates with platelet count of more than 5.5x10^{10}/Unit, 20,(40 %) at day one were analyzed and compared with those with less than 5.5x10^{10}/Unit, 30, (60%) , in the platelet concentrates with platelet count of more than 5.5x10^{10}/Unit ,the mean volume was 76.85±10.49 and the range was between 55-93 ml. The mean white blood cell was 22.41±15.13 and the range was from 0.0-55.3x10^{6}/Unit.

The mean red blood cell was 1.70±0.94 and the range was from 0.0-3.20x10^9/Unit. The mean swirling score was 3, which was expressed by all units. The mean pH value was 8.02±0.19 and range from 7.6-8.2. In the platelet concentrates with platelet count of less than 5.5x10^{10} /Unit the mean WBC count and RBC count were 16.66±17.52 and 1.23±0.55 respectively. The mean platelets concentrates volume, swirling and pH were 68.87±9.22, 2.90±0.31 and 7.90±0.19 respectively. The findings were shown in table (3-3).

3.4 The quality parameters of platelet concentrates at day five:-

The quality parameters measurements of the 50 platelets concentrates were repeated at day five and the results showed a mean platelet count of 4.50±1.46 and range from 1.9-9.2x10^{10}/Unit. Only 30%, (15) of the concentrates had a platelet count of $\geq$ 5.5x10^{10}/Unit .The mean volume was 68.06±10.42 and range from 46-89 ml. The swirling score mean was 1.92±0.63, the range was between 1-3 sore. The mean white blood cells was13.32±13.12x10^6 and the range were from 0.0-57.6x10^6/Unit. Red blood cells mean was 1.33±0.74x 10^9 and the range was 0.0-3.04x10^9/Unit. For pH 47 platelet concentrates were tested and the mean was 7.72±0.23 and range from 7.3-8.1. The findings of day five were shown in table (3-4).

3.5 The effect of storage conditions on platelet concentrates:-

The quality parameters of platelet concentrates at day one were compared with those of day five using the paired t- test. The mean platelet count decreased from 5.09±1.51 at day one to 4.50±1.46 at day five. Also the mean parameters volume, WBCs, RBCs, swirling and pH were decreased on day five than day one. The P value was highly significant for all parameters (0.00). These findings were illustrated in table (3-5) and figures 1,2,3,4,5 and 6.

Table (3-1): ABO and Rh D distribution in platelets concentrates

N=50

B group	Frequency	Percent
A+	8	16%
B+	4	8%
O+	35	70%
O-	3	6%
Total	**50**	**100%**

Table (3-2): Mean ± SD of the quality parameter of platelet concentrates at day 1

	Volume(ml)	PLT count x10^{10}/Unit)	WBCs Count (x10^{6}/Unit)	RBCs Count (x10^{9}/Unit)	Swirling	pH
Mean±SD	72.06±10.42	5.09±1.51	18.96 ±16.69	1.41±0.78	2.94±0.24	7.94± 0.20
Range	50-93	2.2- 9.9	0.0-83.6	0.0-3.20	2-3	7.4-8.2

Table (3-3): Comparison of the concentrates with platelet count of > 5.5 and < 5.5x1010/Unit at day one:-

	WBC count (10^6) Mean ±STD	RBC count (10^9) Mean ±STD	Swirling Mean ±SD	Volume(ml) Mean ±SD	pH Mean ±SD
PLT count >5.5(10^{10})	22.41±15.13	1.70±0.94	3±0.00	76.85±10.49	8.02±0.19
PLT count <5.5(10^{10})	16.66±17.52	1.23±0.55	2.90±0.31	68.87±9.22	7.90±0.19
P value	0.237	0.063	0.083	0.007	0.046

Table (3-4): Mean ± SD of the quality parameter of platelet concentrates at day five.

	Volume	PLT Count x10^{10}/Unit)	WBCs Count (x10^6/Unit)	RBCs Count (x10^9/Unit)	Swirling	pH
Mean±SD	68.06±10.42	4.50±1.46	13.32±13.12	1.33±0.74	1.92±0.63	7.73±0.23
Range	46-89 ml	1.9- 9.2 x10^{10}	0.0-57.6 x10^6	0.0-3.04 x10^9	1-3	7.3-8.1

Table (3-5): Comparison of platelet concentrates at day one and day five of storage.

Variable	Day one (Mean ±STD)	Day five (Mean ±STD)	P value
Volume (ml)	72.06±10.42	68.06±10.42	0.00
PLT count ($x10^{10}$/Unit)	5.09±1.51	4.50±1.46	0.00
WBC count ($x10^{6}$/Unit)	18.9±16.69	13.32±13.12	0.00
RBC count ($x10^{9}$/Unit)	1.41±0.78	1.33±0.74	0.00
Swirling	2.94±0.24	1.92±0.63	0.00
pH	7.94± 0.20	7.73±0.23	0.00

P value significant ($p<0.05$)

P value insignificant ($p>0.05$)

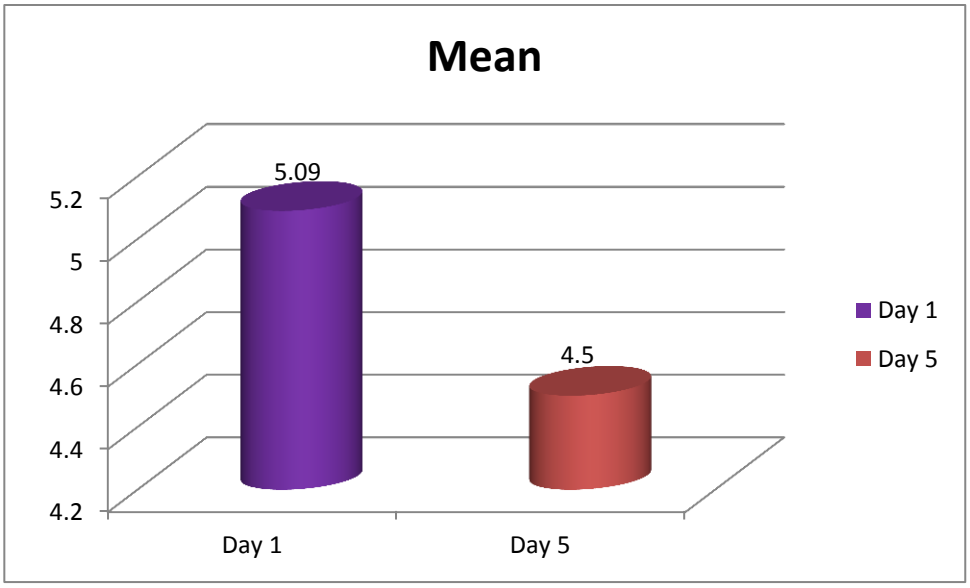


Figure (3-1): Comparison between mean platelet count of platelet concentrates at day one and day five.

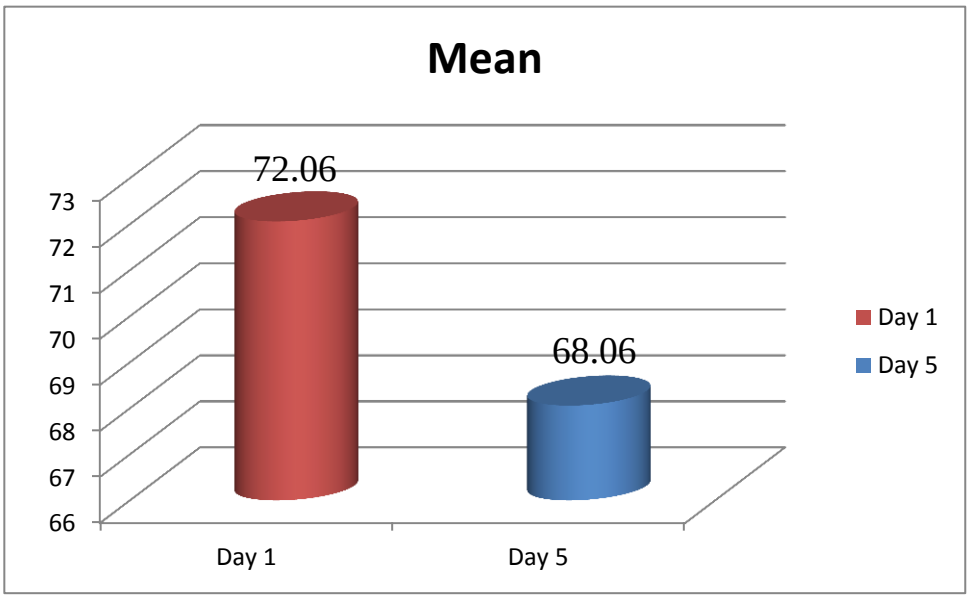


Figure (3-2): Comparison between mean platelet concentrates volume at day one and day five.

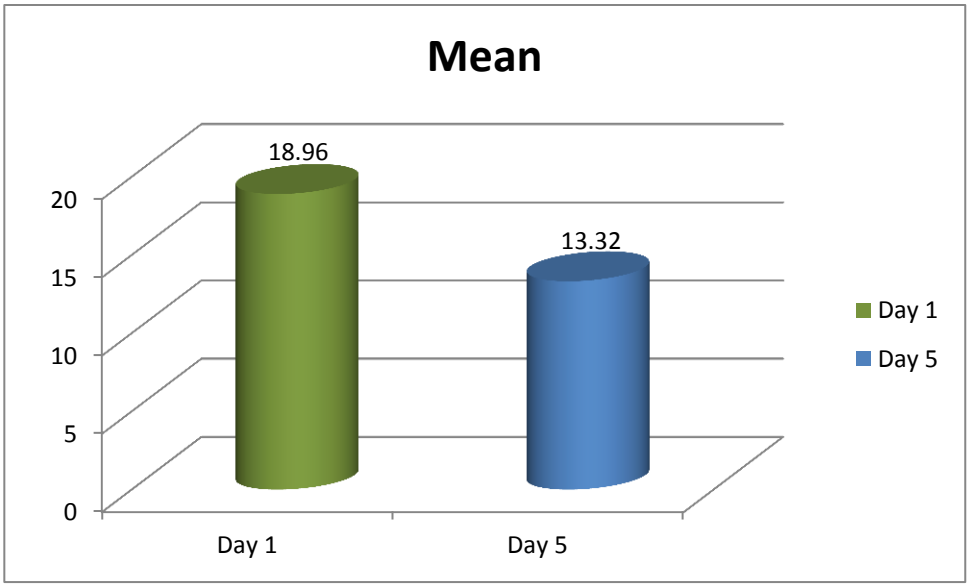


Figure (3-3): Comparison between mean WBCs count in platelet concentrates at day one and day five.

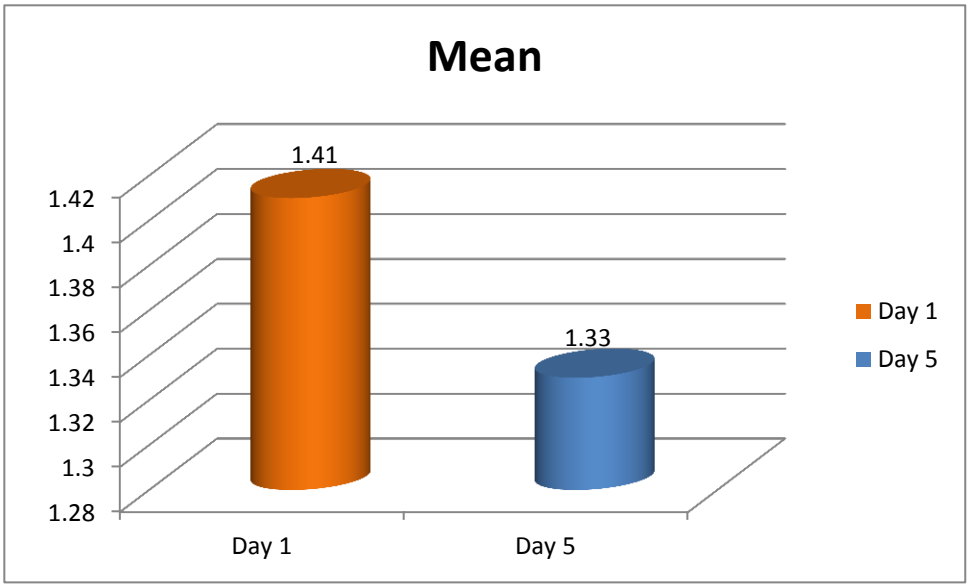


Figure (3- 4): Comparison between mean RBCs count of platelet concentrates at day one and day five.

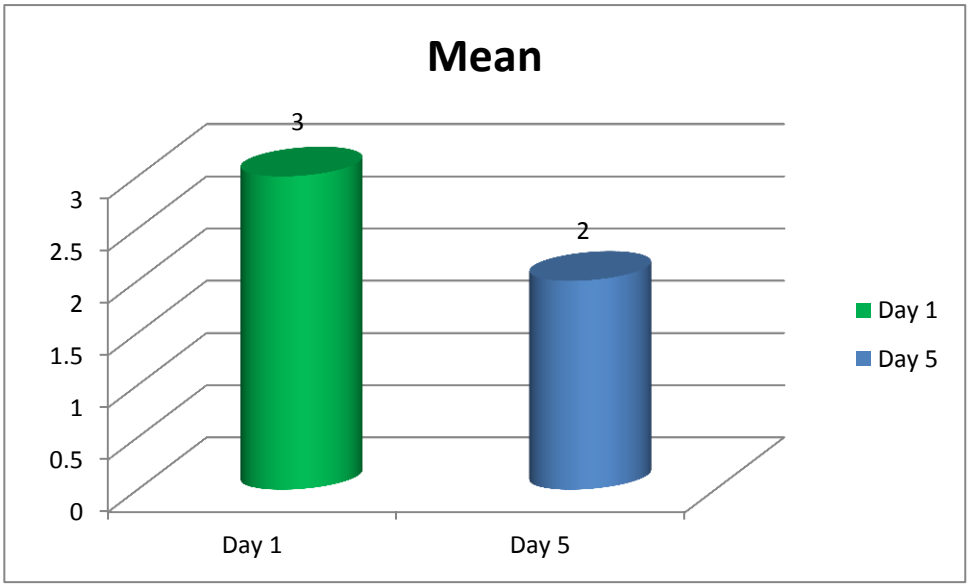


Figure (3-5): Comparison between mean swirling score of platelet concentrates at day one and day five.

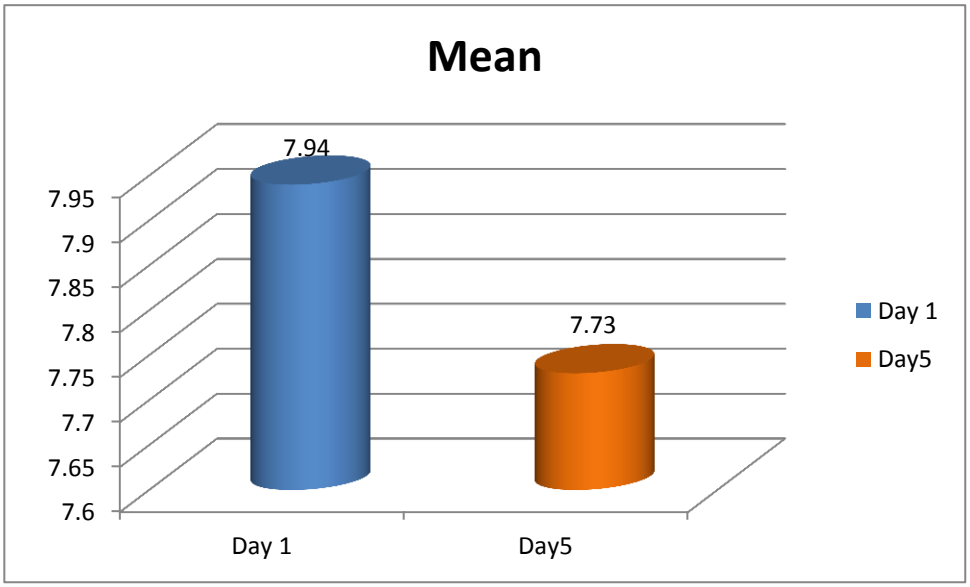


Figure (3-6): Comparison between mean platelet concentrates pH at day one and day five.

Chapter four

Discussion, Conclusion and Recommendations

4.1 Discussion:-

This study was conducted at Elmek Nimer University Hospital Blood Bank and focuses on the assessment of the quality of PCs prepared from whole blood using the platelet rich plasma method on Day 1 and Day 5 to know the effect of collection, preparation and storage conditions on the quality parameters of the platelets concentrates.

A total of 50 platelet concentrates were analyzed. The majority 70%, (35) were group O RhD+, 16%, (8) were group A RhD+, 8%,(4) were group B RhD+ and 6%, (3) were group O RhD- . A total of 47, (94%) of all concentrates were RhD + reflecting that the majority of donors attending the donation department were group O Rh positive .

In this study the quality parameters in term of volume, swirling, platelet count, residual white blood cell , red blood cells and pH were used to assess the quality of platelet concentrates prepared from whole blood using the platelet rich plasma method. The quality parameters of the 50 platelet concentrate units were investigated at day one and day five .On day one the platelet concentrates recorded a volume range from 50-93 ml with a mean of 72.06±10.42. 38%, (19) of the platelet concentrate had a volume of (50-70 ml).The mean platelet count was 5.09±1.51 and range from 2.2-9.9x10^{10} /Unit, 40%, (20) had a platelet count of >5.5x10^{10}/Unit . The residual white blood cells had a mean of 18.96±16.69 and range from 0.0-83.6x10^{6}/Unit. The red blood cells had a mean of 1.41±0.78 and range from 0.0-3.20x10^{9}/Unit. Swirling score had a mean of 2.94±0.24 and range

from 2-3 score. For pH assessment 47 platelet concentrates were tested and the mean pH was 7.94±0.20 and range from 7.4-8.2 .Only 40 %,(20) of the platelet concentrate units had a platelet count of $>5.5x10^{10}$/Unit against AABB standards requirements which stated that ≥ 75 of units should had a platelet count of $>5.5x10^{10}$/Unit . This suggests that approximately more than half of the platelets unit transfusions may not have the expected therapeutic effect on the recipients. The mean platelet count was 5.09±1.51 which was slightly below the minimum threshold. This low platelet count may be due to a defect in preparation procedure particularly centrifugation and separation which should all be revised. In study conducted in the National Transfusion Center (Khartoum) **Khattab *et al*. (2013)** reported that only 50 % of the platelet concentrates had a platelet count of $>5.5\times10^{10}$ /Unit. In similar study conducted at Kenyatta National Hospital in Kenya **Richard *et al*. (2014)** reported that only 51% of all concentrates showed platelet count $>5.5\times10^{10}$ /Unit. The mean and standard deviation for platelet count was $6.63\pm4.73\times10^{10}$ which was well above the minimum threshold. In another similar study at a tertiary hospital in Nigeria where platelet concentrates were prepared by PRP method, **Fasola *et al*. (2002)** found that only 35% of concentrates met the minimum 5.5×10^{10} platelet count threshold. The mean ± SD for that study was 4.17± 3.95. **Singh *et al*. (2009)** reported a mean platelet count value of 7.6± 2.97 which is well above the 5.5×10^{10} threshold in a study in which he assessed PRP, BC and apheresis platelets. These studies demonstrating such wide variations of the means reported for the same method in various regions suggesting variability in platelet production method between regions.

On day five the platelet count was ranged from 1.9-9.2x10^{10}/Unit with a mean of 4.50±1.46 which was significantly lower than day one, the P value was 0.00 . The decrease in platelet count was statistically and clinically significant. This reduction

in platelet count after storage for five days indicates an increase in platelet elimination with storage which could be attributed to platelet senescence, as the platelet's life span is 7-10 days or to platelet storage lesions.

The characteristics of the platelet concentrates with the platelet count of $>5.5\times10^{10}$ /Unit at day one were analyzed and compared with that with platelet count of $< 5.5\times10^{10}$ /Unit. There was no significant difference in residual white blood cells , red blood cells and swirling scores between the two groups, the P value was 0.0.237, 0.063 and 0.083 respectively. The volume and pH were significantly higher in those concentrates with platelet count of $>5.5\times10^{10}$ /Unit than those with $< 5.5\times10^{10}$ /Unit, the P value was 0.007 and 0.046 respectively.

Platelet concentrates made from individual units of fresh whole blood may contain from 0.5×10^8 to 2.5×10^8 WBC/unit (Merryman and Hornblower 1986). Low residual WBC counts in platelet productions are desirable in order to minimize side effects of residual leucocytes such as febrile reactions and to minimize the platelet storage lesion which can be enhanced by high leukocyte count.(Seghatchian and Bessos 1991). Even though leukoreduction was not performed , the residual white blood cells in this study had a mean of 18.96±16.69 and range from 0.0-83.6x10^6/Unit which is considerably lower than the results reported by other studies such as that carried by **Singh *et al*. (2009)** who reported a mean WBC count of $40.5\pm4.8\times10^6$ for the PRP method. **Richard *et al*. (2014)** reported a mean residual white blood cell of ($545\pm429\times10^6$) which is far higher than levels established in this study. These results for WBC count, like those for platelet counts suggest that the marked differences in counts are due to variability in process.

After five days storage the residual white blood cells count in the platelet concentrates was ranged from 0.0-57.6 x10^6/Unit, the mean of it was significantly decreased from18.9±16.69x10^6/Unit at day one to 13.32 x10^6 /Unit, the P value was

0.00. This result was lower from that obtained by **El-Sayed *et al.* (2014)** who reported a mean of 185.50±67.44x10^6/Unit and 144.43±49.51x10^6/Unit at day one and day five respectively.

The contaminated red blood cells in this study had a mean of 1.41±0.78 and range from 0.0-3.20x10^9/Unit. Only 4% (2) of the platelet concentrates did not have detectable red cell contamination which is considerably higher than that reported by **Richard *et al*. (2014)** who reported that only 7.6%, (6) of the concentrates did not have detectable red cell contamination and **Fasola *et al*. (2002)** who found that 30% of concentrates in his study did not have any red cell contamination. The random PCs were prepared from WB by soft-spin to separate the red cells from the PRP and high-spin to separate platelet from the PPP. The presence of red cells in PCs in this study might be caused by the flow of very little amount of red cells into the PCs transfer bag during the first separation process after the soft-spin (first spin). During the hard-spin, the residual red cells may sediment at the bottom together with the platelets. Therefore, the improper separation of PRP from the red cells concentrates after the first spin may cause the presence of residual red cells in the PRP and consequently present of the residual red cells in the PCs.

On day five the mean red blood cells in the platelet concentrates was significantly decreased from1.41±0.78x10^9/Unit to 1.33±0.74x10^9/Unit, the P value was 0.00, this result was lower than that obtained by **Ali (2011)** who reported a mean of 27±2.1 and 24±2.3 at day one and day five respectively.

Platelets prepared from whole blood collections or platelet pheresis are stored in donor plasma, which serves as a buffering agent. PCs from RDPs are typically suspended in 40 to 70 ml plasma to maintain pH. (Holme , Heaton and Moroff 1994).

In this study the platelet concentrate volume was ranged from 50-93 ml with a mean of 72.06±10.42. 38%, (19) of the platelet concentrate had a volume of (50-70

ml) at day one. In study conducted in the National Transfusion Center (Khartoum) **Khattab *et a*l. (2013)** reported that platelets concentrates investigated at day one showed volume range between 43-120ml with a mean of 71ml +/-13.9(54% have a volume of 50-70ml). The mean volume is about the same of that obtained in this study while the range and percent of platelet concentrate with 50-70 ml volume was different. In similar study **Richard *et al*. (2014)** reported a mean volume of platelet concentrates of 74.6 ± 10.7 mls which is nearly similar to the finding in this study. In the study done by **Fasola *et al*. (2002)** the mean volume was considerably low (18.52mls) whereas **Singh *et al*. (2009)** reported a mean of 62. 3 0 ±22.68 ml. Studies have shown that as little as 35-40 mls of plasma is adequate to maintain pH above 6.0, below which the platelet storage lesion is irreversible. (Adam *et al*. 1987).

The volume of PCs was reduced throughout the storage duration because of approximately 4 mL of PCs being taken from the platelet bags for the purpose of platelet count, residual white blood cells, red blood cells and pH determination.

On day five the mean platelet concentrate volume was decreased from72.06±10.42 at day one to 68.06±10.42 at day five. The P value was 0.000 which was highly significant statistically, but it was still clinically accepted.

Evaluation of swirling is a simple noninvasive procedure that can performed a by visual inspection and is useful for routine quality control of each individual PC on a large scale. Visual inspection of swirling correlates with platelet morphology; the presence of swirling indicates discoid morphology and absence is indicative of spherical morphology. (Singh *et al*. 2009). In this study swirling was evaluated at day one and day five and Swirling score had a mean of 2.94±0.24 and range from 2-3 score at day one. Score 3 swirling was observed in 94% , (47) of units and only 6%, (3) revealed score 2 swirling. No unit had score 1 swirling. In similar study **Singh *et al*. (2009)** reported that score 3 swirling was observed in 79.7%, while

score 2 swirling was noticed in 20.3% and no unit had score 1 swirling. Platelet concentrates with score 3 swirling in this study were higher than those reported by **Singh *et al*. (2009)** and also not similar on percent of score 2 swirling while no unit had score 1 swirling in both studies. Findings in this study was also not similar to that reported by **Khattab *et al*. (2013)** who reported that the swirling score was ranged between (+1) – (+3) with a mean of +3 (87 % have score +3).

The mean of swirling scores was significantly decreased from 2.94±0.24 at day one to1.92±0.63 at day five, the P value was 0.000. Score 3 swirling was observed in 47, (94%) of units at day one while on day five only 8(16%) of unit had score 3.

Khattab *et al*. (2013) reported that swirling scores was decreased from 87 % of units had score 3 at day one to only 32% of units had score 3 at day five. A study by **Bertolini (1989)** as cited by Lane (1994). reported that fresh PCs have positive swirling in 83% of units and negative in only 2%, the rest having intermediate swirling. After 5 days of storage, the proportion of PCs with positive swirling decreased to 65% and **Bertolini (1989)** concluded that this drop of swirling could be due to lesions that are known to occur during platelet preservation and hence results of swirling in this study were comparable to reported data.

The pH measurement is considered a global indicator of the platelet environment, demonstrating the balance between platelet metabolism, bacterial contamination if present and the buffer capacity of the medium, with an acceptable range of 6.4 – 7.4 at 22oC in Europe and > 6.2 in USA in order to retain platelet function **(**Yuasa, et al. 2004**).**

In the present study a total of 47 platelet concentrates were tested for pH and the mean pH was 7.94±0.20 and range from 7.4-8.2 at day one. All the PC units had a pH of >6.2.These results are similar to reported values. **Khattab *et al*. (2013)** reported a pH ranged between 6.9-8.2 with a mean of 7.5±0.3 (100 % have a pH > 6.2).

Fasola *et al*. (2002) reported that 100% of his concentrates recorded pH 7.25 or higher while **Singh *et al*. (2009)** reported pH values of 6.7±0.26 (mean ± SD) with a range of 6.5-7.0. In this study when the pH was measured at day five to study the effect of storage conditions the mean was 7.72±0.23 and range from 7.3-8.1 which was statistically significantly lower than pH at day one, P value (0.00) , but it was still clinically accepted. To assure the platelets in the product are viable and functional, the pH must not be acidic and the volume of plasma in which the platelets are suspended must be adequate to keep the pH neutral and allow for gas exchange. (Flynn, 1998). The pH decreases during storage also depending on the stabilizer in plastic platelet storage bags and storage conditions used (Singh, et al. 2009).

4.2 Conclusion

The study revealed that only less than half of the platelet concentrates units fulfilling minimum quality requirement specifications for platelet counts while all units had pH well above quality requirement specifications. Also there was a significant decrease in the quality parameters of the platelet concentrates at day five than day one (P value <0.05) .This indicated that further standardization on the methods of platelet concentrate collections, preparation and storage is required to safe guard adequate and reliable platelet transfusion service.

4.3 Recommendations: -

- The quality of PCs is affected by the preparation method of the PCs, the plastic material of the storage bag, the storage conditions, so that application of proper quality control program that monitor all this steps is essential.
- When platelet concentrates are processed and issued, it is advisable to sample all units and subject them to prerelease quality checks. Implementation of these will help in continuous quality improvement as well as entrench a standardization and harmonization program on platelet quality monitoring.
- Platelet transfusions are an important therapy, and their use will probably continue to increase, so that greater harmonization to the standardized procedures and regulations might promote the use of optimum platelet products and development of the best transfusion policies.
- Yet, studies in therapeutic efficacy in PLT products should be made to promote appropriate transfusion practice.

Chapter Five

References and Appendixes

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جامعة السودان للعلوم والتكنولوجيا

كلية الدراسات العليا

قسم علم الدم

Questionnaire to study the quality parameters of platelets concentrates Prepared at Elmek Nimer University Hospital

التاريخ :-------/-------/-------- مكان التبرع :---------- الرقم المتسلسل ----------

اسم المتبرع :---------- ----------

النوع : ----------ذكر ( )----------انثى ( )----------

العمر : ----------السكن : ----------

رقم الهاتف : ----------

ماهو دافعك اليوم للتبرع : طوعي ( ) لصالح مريض ( )

هل سبق ان تبرعت بالدم ؟ نعم ( ) لا ( )

تاريخ اخر تبرع : ----------/----------/----------

هل تعاني من احد الامراض التالية :

ارتفاع ضغط الدم	نعم ( )	لا ( )
السكري	نعم ( )	لا ( )
امراض القلب	نعم ( )	لا ( )
زيادة تركيز نسبة الدم	نعم ( )	لا ( )
اليرقان (الصفراء)	نعم ( )	لا ( )
هل تعاني من اي مشكلة صحية اخري	نعم ( )	لا ( )
هل تتعاطى اي نوع من الدواء حاليا ؟	نعم ( )	لا ( )
هل تناولت اسبرين خلال 72 ساعة	نعم ( )	لا ( )

السبب ؟ ----------

Laboratory results:

Blood group: ----------

Bag collection time: ----------

Donor platelet count: ----------

Donor TWBCs: ----------

Platelet concentrate results:-

	Volume	**Platelet count**	**Swirling**	**Leucocytes count**	**Red cells count**	**pH**
Day 1						
Day 5						

PLASMA EXPRESSOR SEPARATION STAND , TERUFLEX, ACS-201, TURUMO, Japan).

ROTANTA 460 R, Hettich Centrifuge.

AGITATOR TA-1, SELUTEC GmbH, Germany

TRIPLE BLOOD BAGS