Introduction

Hematopoietic stem cell transplantation (HSCT) is transplantation of multipotent stem cell which has the ability of self-renewal and generation of a functional progeny of highly specialized cells from bone marrow, peripheral blood or umbilical cord to establish marrow and immune function. It is mostly performed for patients with variety of acquired and inherited malignant and nonmalignant disorder including hematologic malignancies (eg, leukemia, lymphoma, and myeloma), nonmalignant acquired bone marrow disorders (eg, aplastic anemia), and genetic diseases associated with abnormal hematopoiesis and function (thalassemia, sickle cell anemia, and severe combined immunodeficiency) ***(Park et al., 2015).***

Georges Mathé, a french oncologist performed the first European bone marrow transplant in 1959 on five Yugoslavian nuclear workers. Later on through 1970s bone marrow derived stem cell transplantation was pioneered by E. Donnall Thomas whose work was later recognized with a Nobel Prize in physiology and medicine ***(Thomas et al., 2004).***

Hematopoietic stem cell transplantation could be either autologous in which patients receive their own stem cells or syngeneic from an identical twin or allogeneic stem cell transplant in which the patient (recipient) receive stem cells from a donor who have human leukocyte-associated antigen (HLA) loci that matches with him ***(Robbie and Deborah, 2010).***

Hematopoietic reconstitution after BM ablation by chemotherapy depends on the migration and "homing" of intravenously transplanted stem cells to the hematopoietic microenvironment in the BM niches of the recipient. HSC "homing" is a multistep process involving sequential activation of adhesion molecules. The chemokine stromal cell-derived factor-1 (SDF-1) was the first identified chemo-attractant for monocytes, lymphocytes, and $CD34^+$ cell homing ***(Hatzimichael and Tuthill, 2010).***

Both autologous and allogeneic HSCT are used depending on donor availability, the patient's medical condition, the therapeutic objectives, and the availability and source of stem cells ***(Hatzimichael and Tuthill, 2010).***

An important barrier to allogeneic hematopoietic stem cell transplantation is the availability of suitable donor. The

source of donated stem cells (the donor) may be genetically related or unrelated to the recipient according to the degree of human leukocyte antigens (HLA) match between the donor and the recipient; well-matched transplants decrease risks of graft rejection and graft versus host disease (GVHD), both of which are among the most serious sequelae of transplantation ***(Barrett, 2009).***

The major advantages of an allogeneic graft include the absence of malignant cells contaminating the graft, the potential for an immunologic anticancer graft- versus tumor effect, and the ability to treat malignant and nonmalignant disorders of the bone marrow including genetic and immunologic diseases. While disadvantages include the difficulty in finding an appropriate HLA-matched donor, and development of GVHD after HCT which contributes to high morbidity and mortality of the procedure ***(Nakamura and Forman, 2015).***

In autologous transplant, the re-infused stem cells come from either the patient's own bone marrow or peripheral blood. These cells do not cause GVHD, and thus, autologous transplant is associated with less morbidity and mortality than allogeneic HCT and increases in the number

of patients who can undergo the procedure in the upper age limit. Whereas disadvantages include the possibility of tumor cell contamination within the graft in many diseases which can cause relapse, the lack of a significant therapeutic graft versus tumor effect, and the limited ability to use autologous stem cells to treat patients not in remission or with inherited non-malignant hematopoietic diseases ***(Nakamura and Forman, 2015)***

Hematopoietic stem cells can be obtained directly from bone marrow by multiple aspirations from the anterior and posterior spines of the pelvis, the upper sternum in adults, and the head of the tibia in infants while the patient is under general anesthesia. Alternatively, hematopoietic stem cells can be obtained from peripheral blood, after stimulation with hematopoietic growth factors such as granulocyte colony-stimulating factor (G-CSF) followed by leukapheresis, or they can be obtained from cord blood sources ***(Cutler and Ballen, 2009).***

Peripheral blood stem cells (PBSC) are now the most usual source of stem cells, whereas until about 10 years ago BM was virtually always used. There are advantages and disadvantages to each method of donation. The use of cord

blood transplantation has rapidly increased due to favorable factors including ease of collection, expanded and prompt availability, no risk to the donors, a decreased risk of adverse effects (eg: GVHD, transmission of infections), and increased tolerance to HLA-mismatch ***(Kulkarni and Treleaven, 2009).***

Although; high doses of chemotherapy used in HSCT cause significant drug toxicities and complications from prolonged immunodeficiency and require an extended recovery process, the recognition of risk factors for complications allows the design of risk-specific supportive-care regimens that reduce the incidence of transplantation morbidity and mortality ***(Hatzimichael and Tuthill, 2010).***

Recently, efforts to diminish GVHD while forcing graft-versus-tumor effects, and advances in prophylaxis and treatment of cGVHD will remain of high priority. Changes in stem cell sources and graft manipulation and new developments in donor selection might have additional influence on immune reconstitution and post-transplant disorders of infectious and immunological type with more success in HSCT ***(Halter et al., 2012).***

Aim of work

The aim of this work is to:

- Asses the role of CD73 in development and severity of graft-versus-host disease in patients undergoing allogeneic hematopoietic stem cell transplantation (HSCT) due to different types of hematological disorders.

Hematopoietic stem cell Transplantation

Sources of HSCT and storage:

1- Bone marrow: In case of bone marrow transplantation (BMT) hematopoietic stem cells are obtained from anterior and posterior iliac crests, upper sternum in adults and head of tibia in infants through a large needle that reaches the center of the bone. Multiple aspirations from these sites rupture marrow sinusoids allowing marrow cells to be aspirated in a mixture of venous blood, a process that lasts1-2 hours ***(Burt et al., 2008).***

The harvested marrow is filtered, stored in a special solution bags and then frozen. When the marrow to be used, it's thawed and then given just like a blood transfusion. The stem cells travel to the recipient's bone marrow and begin to make new blood cells(engraft) which can be measured in the patient's blood tests in about 2 to 4 weeks ***(Bishop and Pavletic, 2008).***

2- Peripheral blood stem cells (PBSC): Normally, Circulating hematopoietic stem cells (HSC) presents at frequencies 1/100 to1/1000 times lower than they are in the marrow, granulocyte colony-stimulating factor (G-CSF) mobilizes

HSC to peripheral blood from which they are collected by aphaeresis, PBSC collections are heavily mixed with peripheral blood cells and contain about 10-fold more lymphocytes than do BM collection and used for both allogeneic and autologus HSCT and it's the most common source used nowadays ***(Childs, 2011).***

After patients' treatment with chemo and/or radio-therapy (conditioning process) the stem cells are given by infusion. The new cells are usually found in the patient's blood a few days sooner than bone marrow sources, usually in about 10 to 20 days ***(Delaney et al., 2010).***

3- Umbilical cord blood (UCB): Around 30% of unrelated hematopoietic stem cell transplants are done with cord blood which obtained when a mother donates her placenta and/or infant's umbilical cord after birth ***(Spellman et al., 2012).***

At birth, the umbilical vein is rich in HSC but the total volume is usually low less than 100 ml. Cord blood stem cells have strong proliferative potential which partly compensates for the very much lower volume. UCB lymphocytes are largely naive (non-antigen experienced) but have strong proliferative potential; still, cord blood

transplantation can take longer time to engraft ***(Delaney et al., 2010).***

However, umbilical cord blood transplantation more suitable for small children and younger adults. Researchers are now looking for ways to use cord blood in larger adults either by increasing the numbers of stem cells in the lab before transplantation (ex-vivo expansion of cord blood units), or by using cord blood from 2 infants at the same time for one adult called a *dual-cord-blood transplant* ***(Gratwohl et al., 2010).***

So all of these 3 sources of stem cells are used for the same goal but each of them may have some pros and cons as shown in table (1) ***(Magenau et al., 2011).***

Table (1): Shows comparison between different sources of stem cells transplant ***(Magenau et al., 2011).***

	Cord blood	**PB-HSCT**	**BM-HSCT**
Collection	Placental blood	G-CSF mobilization	Multiple aspiration
HSC minimum for graft×106/kg	1.0	1.0	0.1
Neutrophils >500/µl median days post SCT	14	12	21
Platelets >20,000/µl median days post SCT	21	18	28
Risks for the donor	None	Yes	Yes
Factors limiting engraftment	Cell count	HLA matching	HLA matching
Factors affecting the outcome	Engraftment failure Delayed immune-response	GVHD	GVHD
Risks for GVHD	Low	High	High
Acute GVHD	Low	High	High
Chronic GVHD	Low	High	High
Post-transplant infections	Higher	High	High

Donors for allogeneic transplant may be ***related*** (usually a closely HLA matched sibling), ***syngeneic*** (a monozygotic or 'identical' twin of the patient, its main advantage is that GVHD will not occur due to perfect HLA matching, on the other hand disadvantages including it

is extremely rare, and it won't help to destroy the remaining cancer cells as the new immune system is much like the recipients' one) or ***unrelated*** (donor who is not related to the patient, and found to have very close degree of HLA matching) ***(Nivison et al., 2005).***

The HLA antigen (human leukocyte antigen) is a gene complex encoding the major histocompatibility complex (MHC) proteins in humans. These cell-surface proteins are responsible for the regulation of the immune system. It's located on chromosome 6, covers about 3600 kilobases of DNA, and contains in excess of 120 expressed genes. Two classes of HLA molecules are central to the control of immune responses: class I (A, -B, -C) and Class II (DR, -DQ, -DP) (Figure1.1). Class I molecules are heterodimorphic glycoprotein whose alpha chain (~45 kD) is encoded by HLA-complex genes (*HLA-A, -B, C*) and beta chain is β2 microglobulin (β2-M) encoded on chromosome 15 ***(Iqbal et al., 2012).***

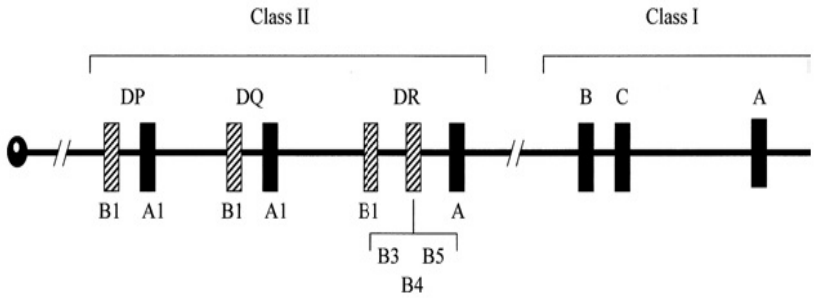

Figure (1): HLA class I and class II gene of HLA complex. This figure shows an abbreviated representation of HLA region of chromosome 6. Solid bars represent α chain gene and slashed bar represent β chain genes. When present, the DRB3*, DRB4*, DRB5 genes are located between DRA1* and DRB1* genes.

Class II molecules are also heterodimeric glycoprotein, but both alpha and beta chains (~28–32 kD) are encoded within the HLA complex (alpha genes *HLA-DRA1*, -DQA1*, -DPA1** and beta genes *HLA-DRB1*, -DRB3*, -DRB4*, -DRB5*, -DQB1*, -DPB1**) ***(Schreuder et al., 2005).***

The routinely tested antigens before stem cell transplantation are Class I and class II HLA antigens. At least 8 HLA markers are done: two A, two B, two C, and two DRB1 markers and sometimes DQ, to match. An adult donor must match at least 6 of these 8 HLA markers and many transplant centers require at least a 7 of 8 matches ***(Sirinoglu et al., 2012).***

The proteins encoded by HLAs are those on the outer surface of body cells that are (effectively) unique to that

person, antigen presenting cells and other proteins involved in the immune system. The immune system uses the HLAs to differentiate self cells and non-self cells ***(Erlich et al., 2011).***

Searching for unrelated donor should start as soon as matched related donor isn't available. The largest donor registry are the cord blood banks and NMDP (National Marrow Donor Program), which is responsible for providing marrow and stem cells for more than 9000 recipients to date. Bone Marrow Donors Worldwide (BMDW) is a collective database of 66 registries in 49 countries and 47 cord blood registries from 31 countries making it the largest database in the world ***(Halter et al., 2012).***

On the other hand, autologus HSCT requires extraction of hematopoietic stem cells and lymphocytes from the patient himself and stored frozen in liquid nitrogen (for years if necessary) and retain their viability on thawing. Cryopreservation requires slow, controlled-rate freezing with addition of an agent (usually dimethyl sulfoxide – DMSO) which prevents intracellular ice crystal cell damage. Patients are then treated with high dose chemotherapy with/ without radiotherapy, when the cells are required for

transplantation they must be rapidly thawed, and transfused to minimize DMSO toxicity ***(Barrett, 2009).***

Autologus transplantation has the advantage of lower risk of infection during the immune-suppression period. Also, the incidence of graft-versus-host disease is very rare. These advantages have established autologus HSCT as one of the standard second-line treatments for such diseases as lymphoma ***(Bruno et al., 2007).***

Tandem transplants which also called double autologus transplant. In this technique; the patient gets 2 courses of high-dose chemotherapy, each followed by transplantation of their own stem cells ***(Magenau et al., 2011).***

All of stem cells needed are collected before the first high-dose chemo therapy, and half of them are used for each transplant. Most often both courses of chemotherapy are given within 6 months, after patients' recovery from the first one ***(Kumar et al., 2009).***

Eligibility and indications of different types of HSCT:

Eligibility for both allogeneic and autologus HSCT varies across countries and institutions. Ultimately, decisions

regarding transplant eligibility have been made on a case by case basis based on a risk-benefit assessment, needs and wishes of the patient ***(Elstrom et al., 2010).***

In general auto-HSCT may be considered in the following conditions:

- **Hodgkin lymphoma**: High-dose chemotherapy and autologous HSCT are the treatments of choice for patients with poor prognosis, early relapse after initial chemotherapy (less than 12 months), resistant disease, or second relapse ***(Rzepecki et al., 2009).***
- **Multiple myeloma**: the treatment options of multiple myeloma are complex due to rapid advances in stem cell transplantation, newer medications, and better supportive care which have led to better survival over the past 30 years. The main options for treatment include non-chemotherapy drugs (immunomodulatory drugs, proteasome inhibitors, and monoclonal antibodies), standard chemotherapeutic drugs, corticosteroids, and stem cell transplantation (autologous more commonly used than allogenic) ***(Kumar et al., 2014).***