Role of cytokines genes polymorphisms in predicting the outcome of HLA matched sibling bone marrow transplantation

Thesis

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By

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Abstract

Graft versus host disease (GVHD) is the major complication of allogeneic hematopoietic stem cell transplantation (HSCT) and cytokines are recognized as important mediators of GVHD. Polymorphisms in the regulatory regions of several cytokine genes have been associated with a number of immune diseases as well as post HSCT complications. The aim of this study was the detection of cytokines genes polymorphisms (CGPs) and their relation to post HSCT complications mainly GVHD in Egyptian patients receiving HSCT. The study included 106 patient/donor pairs who received peripheral blood stem cell transplantation (PBSCT) from HLA-matched identical siblings. DNA for cytokine genotyping was available for 105 patients and 98 donors only. Polymerase chain reaction with sequence specific primers (PCR-SSP) technique was used to analyze the following cytokines genotypes: Tumor necrosis factor-alpha (TNF-α) (−308), transforming growth factor beta1 (TGF-β1) (codon10 and codon 25), interleukin (IL)-6 (−174), IL-10 (−1082, −819 and −592) and Interferon –gamma (IFN-γ) (+874). From the current study we can conclude that there was no impact of all tested CGPs on development of GVHD or other post HSCT complications.

**Key words:** Allogeneic HSCT, Post HSCT complications, GVHD, CGPs, PCR-SSP.
Introduction and Aim of the work

Introduction

Hematopoietic stem cell transplantation (HSCT) is one area of stem cell research with major advances in the cure of hematological disorders such as leukemia and lymphoma. The overall survival rate after HSCT is only 40-60%. The cure of such patients is hampered by clinical complications that arise post-transplant. These are largely the result of a lack of understanding of acceptance and rejection mechanisms and genetic differences that exist between a given patient and donor. These differences include transplantation antigens [major histocompatibility antigen and minor histocompatibility antigens (mHag)] and, non-human leukocyte antigen (HLA) functional gene polymorphisms, which can result in a greater risk and severity of transplant-related complications. In addition, many donor-, recipient-, disease- and transplant related factors (such as age, gender, cytomegalovirus (CMV) serologic status, disease type, conditioning regimen or hematopoietic stem cell source and dose), can affect occurrence and severity of HSCT complications (Dickinson et al., 2004).

Although HLA compatibility remains the central means of selecting donors, the sequencing of the human genome has revealed numerous non-HLA encoded single nucleotide polymorphisms (SNPs) whose significance in allogeneic HSCT has to be investigated. The polymorphisms within the regulatory sequences of genes may alter, for example, the amount of cytokine produced and the degree of drug metabolizing enzyme activity (Dickinson et al., 2004).
Acute graft versus host disease (GVHD) incidence following allogeneic HSCT from HLA identical donor siblings is 30–80% and can be fatal in up to 50% of cases (Reddy and Ferrara, 2003).

The release of pro-inflammatory cytokines interleukin-1 (IL-1), IL-6, IL-8 and tumor necrosis factor-alpha (TNF-α) during radiation and chemotherapy conditioning regimens is involved in the activation of antigen presenting cells (APCs), apoptotic damage of GVHD target tissues, induction of donor T-cell activation, and up-regulation of recipient HLA and adhesion molecule expression. Accelerated tissue damage then ensues from activated T cells and natural killer (NK) cells with release of predominantly T-helper 1 (Th1) type cytokines namely IL-2, interferon gamma (IFN-γ) and TNF-α resulting in a ‘cytokine storm’. Although T cells are central to the initiation of GVHD, proinflammatory mediators, such as IL-1 and TNF-α, are alone capable of inducing the pathological changes of GVHD. Taken together it is evident that the genetic make-up of the recipient and the donor can strongly influence the success or failure of HSCT (Reddy and Ferrara, 2003).

Many of the reported cytokine gene polymorphisms (CGPs) occur within apparent regulatory regions of the gene. Within normal populations, high or low producers of cytokines naturally exist because of the inherited gene polymorphisms. Initial studies within the solid organ transplant setting demonstrated that patients with high producer TNF and low producer IL-10 genotypes were more likely to reject their solid organ graft (Turner et al., 1995; 1997-a, b). These studies have been extended to the HLA-matched sibling and unrelated HSCT settings, and a number of other CGPs have been associated with GVHD and HSCT transplant outcomes (Stark et al., 2003; Keen et al., 2004).
The TNF-α gene is located within the class III region of the major histocompatibility complex (MHC). Therefore, in an HLA-identical sibling HSCT, the recipient and donor genotype will be identical and may equally or additively affect TNF production and HSCT outcome. Studies have investigated the role of the TNF-α (-308) in HSCT and GVHD, but results have been inconsistent. TNF-α (-308) in HLA-matched sibling transplants was initially based on TNF-α secretion studies, not confirmed in larger cohorts (Dickinson et al., 2008).

Several studies have reported association of Transforming growth factor-beta (TGF-β) genotype with GVHD. A polymorphism (-509 C/T) in the promoter region is associated with variation in plasma concentration of TGF-β (C allele with higher production), and amino acid substitutions at codons 10 (Leu→Pro) and 25 (Arg→Pro) which alters protein structure (Dickinson et al., 2004). Leffell et al., have reported an influence of the high expression G/G genotype for TGF-β codon 25 with more severe GVHD (Leffell et al., 2001).

The SNPs and microsatellites of the IL-10 gene resolve into several conserved haplotypes. Three common haplotypes of the promoter region that lie between -1082 and -592 represent high, intermediate and low (GCC, ATA, ACC) production of IL-10. The low producer (ACC) haplotype in the recipient was associated with severe acute GVHD (grades III–IV) in cyclosporine (CSA) alone (Cavet et al., 1999) and CSA plus Methotrexate (MTX) (Ishikawa et al., 2002) treated HLA-matched sibling HSCT cohorts. The IL-10 low producer haplotype has been confirmed to play a role in both survival and GVHD (grades III–IV) in HLA-matched sibling transplants in two separate large cohorts (>400 patients) (Lin et al., 2003).
Possession of the IL-6-174 polymorphism G allele in the recipients of HLA-matched sibling transplants has been associated with both acute and chronic GVHD (Cavet et al., 1999; Socie´ et al., 2001-b) and this allele has also been correlated with high serum IL-6 levels in normal individuals (Dickinson et al., 2004).

The SNPs and microsatellite polymorphism were detected in the first intron of the IFN-γ gene at position +874 (T/A). Allele 2 of the CA repeat microsatellite in intron 1 has been associated with high in vitro IFN-γ production. Possession of allele 3 in the recipient genotype in HLA-matched sibling transplants was associated with development of acute GVHD (Cavet et al., 2001) and chronic GVHD (Bogunia-Kubik et al., 2005).

Aim of the work

Detection of cytokine gene polymorphisms and their relation to post transplant GVHD in Egyptian Patients receiving HSCT from an identical sibling.

Patients and Methods

Patients: All Donors and Recipients who receive HLA matched sibling stem cell transplantation at (Nasser Institute, Ministry of Health) during one year.

Inclusion criteria: All patients receiving allogeneic HSCT.

Exclusion criteria: Early death before 3 months.

The following genetic polymorphisms will be tested:

1- TNF-α (-308) (G/A) genotype.

2- TGF-β1 (codon 10 and 25) genotype.
3- **IL-10**: Three common haplotypes of the promoter region that lie between (1082 and 592) represent high, intermediate and low (GCC, ACC, ATA) IL-10 producer alleles.

4- **IL-6**: -174 (G/C) genotype

5- **IFN-γ**: +874 (T/A) genotype

Testing for the different polymorphisms will be performed using specific primers and standard PCR amplification (*Cavet et al., 1999; Lin et al, 2003*).

Informed consent will be taken from all recipients and donors according to protocols approved by ethical committee at National Cancer Institute (NCI).

Data concerning recipient age, donor age, sex, diagnosis and disease status will be retrieved from patients’ files.

The patients will be followed up for at least 6 months from transplantation and will be examined for the development of GVHD which will be diagnosed and graded according to standard criteria (*Przepiorka et al., 1995*).

The detected CGP will be related to different prognostic factors and post transplant GVHD.

**Statistical method**

Data will be analyzed using SPSS statistical package version 16.

The relationship between SNPs genotypes and development of GVHD will be done using log rank test.

All tests will be two sided, p-value less than 0.05 will be considered significant.
Hematopoietic stem cell transplantation

Introduction

Hematopoietic stem cell transplantation (HSCT) involves the intravenous infusion of hematopoietic stem (progenitor) cells collected from bone marrow (BM), peripheral blood (PB), or umbilical cord blood (UCB) to reestablish hematopoietic function in patients whose BM or immune system is damaged or defective (Armitage, 1994). The hematopoietic stem cells (HSCs) can be obtained either from a genetically identical twin (syngeneic) or from an HLA-identical matched sibling or matched unrelated donor (allogeneic) or from the patient’s own (autologous) BM or PB (Bedi et al., 2003).

HSCT is performed as part of therapy to eliminate a BM infiltration such as leukemia or to correct congenital immunodeficiency disorders. Also, HSCT is used to allow patients with cancer to receive higher doses of chemotherapy than BM can usually tolerate; BM function is then salvaged by replacing the marrow with previously harvested stem cells (Perumbeti et al., 2014).

More than 28,000 autologous transplantation procedures and 21,000 allogeneic transplantation procedures are performed every year worldwide, per the first report of the World Wide Network of Blood and Marrow Transplantation (Gratwohl et al., 2010). The number continues to increase by 10-20% annually, and reductions in organ damage, infection and severe acute graft versus host disease (GVHD) seem to be contributing to improved outcomes (Gooley et al., 2010).

Thomas was a pioneer in applying the results from early studies in animals to the treatment of leukemia in people. In 1959, he and his colleagues reported that a patient with end-stage leukemia who was treated with total-body irradiation (TBI), followed by infusion of her
identical twin’s marrow, had a three-month remission (Thomas et al., 1959). Allogeneic transplantation became feasible in the early 1960s, after the identification and typing of HLA. The genes for HLA are closely linked on chromosome 6 and are inherited as haplotypes. Thus, two siblings have about one chance in four of being HLA identical. Transplantation of BM from an HLA-matched child to his immunodeficient sibling was successful because the recipient could not reject the allograft (Gatti et al., 1969).

HSCT program in Egypt started in 1989 on a narrow scale. In 1997, the transplant rate increased dramatically with the opening of the bone marrow transplantation (BMT) unit at Nasser institute. The total population in Egypt in 2014 is 90 million with 10 transplant centers performing about 580 transplants per year. HSCT rate in Egypt compared to other countries are shown in table (1) (Mahmoud, 2015).

**Table (1): HSCT rate in Egypt in comparison to other countries (Mahmoud, 2015)**

<table>
<thead>
<tr>
<th>Country</th>
<th>Population (millions)</th>
<th>Centers</th>
<th>Transplants per year</th>
<th>Rate/million</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egypt</td>
<td>90 m</td>
<td>10</td>
<td>580</td>
<td>6.4</td>
</tr>
<tr>
<td>France</td>
<td>57.6 m</td>
<td>45</td>
<td>2454</td>
<td>42.6</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>57 m</td>
<td>46</td>
<td>1736</td>
<td>30.1</td>
</tr>
<tr>
<td>Italy</td>
<td>58 m</td>
<td>54</td>
<td>2115</td>
<td>36.7</td>
</tr>
<tr>
<td>Germany</td>
<td>81 m</td>
<td>54</td>
<td>2400</td>
<td>29.6</td>
</tr>
</tbody>
</table>

The main HSCT centers in Egypt include: Nasser institute and Sheikh Zayed hospitals, Kasr El-Ainy hospital, Abou El-Reesh hospital, Ain Shams University, Alexandria University, Mansoura University, Military force hospital, Dar El-Fouad hospital, Wadi El-Nil hospital and
International medical center (Mahmoud, 2015). The BMT unit at NCI, Cairo University is temporarily closed because of renovations.

**Indications for HSCT**

A number of malignant and non malignant diseases can be treated by allogeneic and autologous HSCT (Table 2) (Copelan, 2006). HSCT has led to the cure of diverse forms of cancer, BM failure, hereditary metabolic disorders, hemoglobinopathies, and severe congenital immunodeficiencies that would otherwise have been fatal (Perumbeti et al., 2014).

The indications for HSCT vary according to disease categories and are influenced by factors such as cytogenetic abnormalities, response to prior therapy, patient's age, disease status (remission versus relapse), availability of a suitable graft source, time of referral and time to transplant (Perumbeti et al., 2014).

Acute myeloid leukemia (AML) constitute about 26%, BM aplasia 23%, Chronic myeloid leukemia (CML) 18%, Acute lymphoblastic leukemia (ALL) 10% of yearly allogeneic transplants in Egypt, while autologous transplants are performed mainly for lymphomas (Hodgkin’s disease (HD) 24%, Non-Hodgkin’s lymphoma (NHL) 22%) and Multiple myeloma (MM) 24% (Mahmoud, 2015).

**Table (2): Common indications of Hematopoietic Stem Cell Transplantation (Copelan, 2006)**

<table>
<thead>
<tr>
<th>Autologous transplantation</th>
<th>Allogeneic transplantation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malignant disorders</td>
<td>Malignant disorders</td>
</tr>
<tr>
<td>Non-malignant disorders</td>
<td>Non-malignant disorders</td>
</tr>
<tr>
<td>Multiple</td>
<td>Autoimmune</td>
</tr>
<tr>
<td></td>
<td>Acute myeloid</td>
</tr>
<tr>
<td></td>
<td>Severe aplastic</td>
</tr>
<tr>
<td>myeloma (MM)</td>
<td>disorders*</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>Non-Hodgkin’s lymphoma (NHL)</td>
<td>Amyloidosis</td>
</tr>
<tr>
<td>Hodgkin’s disease (HD)</td>
<td>Chronic myeloid leukemia (CML)</td>
</tr>
<tr>
<td>Acute myeloid leukemia (AML)</td>
<td>Myelo-dysplastic syndromes (MDS)</td>
</tr>
<tr>
<td>Neuroblastoma (NB)</td>
<td>Myeloproliferative disorders (MPD)</td>
</tr>
<tr>
<td>Ovarian cancer</td>
<td>Non-Hodgkin’s lymphoma</td>
</tr>
<tr>
<td>Germ-cell tumors</td>
<td>Hodgkin’s disease</td>
</tr>
<tr>
<td></td>
<td>Chronic lymphocytic leukemia (CLL)</td>
</tr>
<tr>
<td></td>
<td>Multiple myeloma</td>
</tr>
</tbody>
</table>

*Autoimmune disorders as systemic lupus erythematosus (SLE) and systemic sclerosis

**Indications for HSCT in malignant diseases**

**Acute myeloid leukemia (AML) (table 3)**

Following induction chemotherapy 60%–75% of patients with AML achieve complete remission (CR). This is followed by 2–4 cycles of consolidation chemotherapy. Patients with favourable cytogenetic findings such as those with t(15;17), t(8;21), and inv 16 or del 16 have 5-year overall survival (OS) rates close to 50% with chemotherapy alone and do not appear to benefit from HSCT. Patients with poor risk cytogenetics such as those with t(8;21) with del 9q, inv (3q), abnormal
11q23, 20q, 21q, del 9q, t(6;9), t(9;22) or complex karyotypes (≥3 abnormalities) have poor CR and should be considered for allogeneic HSCT. Autologous HSCT could be considered in AML patients in first complete remission (CR1), poor risk category if HLA-matched sibling donor is not available. Patients with intermediate risk cytogenetics such as +8, −Y, +6 and del 12 are indicated for HSCT if an HLA-identical sibling is available. For patients with normal cytogenetics, allogeneic HSCT should be considered if the patient requires 2 cycles to achieve CR. Patients in the second complete remission (CR2) or those with an untreated relapse can be cured with allogeneic HSCT. About 10%–20% of patients with primary chemo-refractory AML can be salvaged with allogeneic HSCT (O’Donnell, 2004).

Acute lymphoblastic leukemia (ALL)

About 80% of children with good risk ALL are cured with standard chemotherapy. Results with autologous HSCT are not superior to chemotherapy alone in patients with high risk ALL. Allogeneic HSCT is recommended in high-risk ALL for patients who have various chromosomal abnormalities (Kumar, 2007).


<table>
<thead>
<tr>
<th>Group</th>
<th>Allogeneic HSCT</th>
<th>Autologous HSCT</th>
</tr>
</thead>
<tbody>
<tr>
<td>AML, in first complete remission (CR1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Good cytogenetics: t(8;21); inv 16</td>
<td>Not indicated</td>
<td>Not indicated</td>
</tr>
<tr>
<td>Normal cytogenetics</td>
<td>Not indicated</td>
<td>Possibly not indicated</td>
</tr>
<tr>
<td>Poor cytogenetics</td>
<td>Indicated</td>
<td>If no match, then indicated</td>
</tr>
<tr>
<td>AML, in second complete remission (CR2)</td>
<td>Indicated</td>
<td>If no match, then indicated</td>
</tr>
<tr>
<td>----------------------------------------</td>
<td>-----------</td>
<td>----------------------------</td>
</tr>
<tr>
<td>AML relapse</td>
<td>Indicated</td>
<td>Not indicated</td>
</tr>
<tr>
<td>Refractory AML</td>
<td>Indicated</td>
<td>Not indicated</td>
</tr>
</tbody>
</table>

Allogeneic HSCT is usually indicated for (Kumar, 2007):

1. Children <15 years of age with cytogenetic abnormalities such as t(4;11) and Philadelphia (Ph) chromosome, t(9;22).
2. Children in CR2 or third complete remission (CR3).
3. Young adults between 15 and 21 years of age who have a high leukocyte count at diagnosis and have the Ph+ chromosome.
4. For adult patients with Ph+ ALL, early allogeneic HSCT from a sibling donor is the treatment of choice.

HSCT may be also a good option for patients who have experienced induction failure (Satwani et al., 2007) and patients with early relapse within 18 months of diagnosis (Bailey et al., 2008; Nguyen et al., 2008).

**Chronic myeloid leukemia (CML)**

Tyrosine kinase (TK) inhibitor (Imatinib mesylate) is the treatment of choice for all newly diagnosed CML patients especially with positive Philadelphia chromosome. It achieves complete cytogenetic response in 50%–75% of patients at 12 months (Goldman and Melo, 2003).

Allogeneic HSCT is considered for (Kumar, 2007):

1. Patients fail to achieve complete hematological remission after 3 months of imatinib therapy.
2. Patients fail to achieve complete cytogenetic response after 12–18 months of imatinib therapy.
3. Patients relapse after an initial response.
4. Patients with advanced disease (accelerated phase/blast crisis).
5. Younger patients <30 years of age.

Alternatives to imatinib, such as dasatinib, have extended spectra of activity against the Breakpoint cluster region/Abilson (BCR/ABL) fusion product and may rescue some patients who have failed or lost response to imatinib (Cortes et al., 2008).

**Chronic lymphocytic leukemia (CLL)**

Although HSCT is the treatment of choice for many aggressive hematologic malignancies, the role of HSCT in CLL has remained controversial. Despite an improved disease-free survival (DFS) in some patients, multiple, prospective, randomized autologous HSCT trials failed to demonstrate an OS benefit as compared to conventional therapy. Allogeneic BMT can successfully eradicate CLL with adverse prognostic features. In the older CLL patients, non-myeloablative allogeneic transplants are better tolerated than myeloablative transplants (Gladstone and Fuchs, 2012).

**Myelo-dysplastic syndrome (MDS)**

Allogeneic HSCT is the treatment of choice for patients with International Prognostic Scoring System (IPSS) intermediate-2 and high risks MDS. However, its use is limited because of the higher median age of patients at the time of diagnosis (70 years). Autologous HSCT can be considered in selected patients who achieve CR following induction chemotherapy and do not have an HLA-identical donor (Kumar, 2007).

**Non-Hodgkin lymphoma (NHL)**

A combination of high-dose chemotherapy and autologous or allogeneic HSCT has produced complete remissions in patients with relapsed disease and in patients who have not achieved complete remission with primary therapy (Rzepecki et al., 2009).
Hodgkin disease (HD)

High-dose chemotherapy and autologous HSCT are the treatments of choice for patients with poor prognosis and early relapse after initial chemotherapy or induction failure (Cashen and Bartlett, 2008). The role of allogeneic transplant in refractory or relapsed, chemo-resistant HD is still under investigation (Perumbeti et al., 2014).

Multiple myeloma (MM)

Initial induction therapy with thalidomide–dexamethasone followed by autologous PB HSCT is considered the standard treatment for myeloma patients ≤65 years of age (Kumar et al., 2006).

Neuroblastoma (NB)

Autologous transplantation is the main therapy for patients with high risk NB in CR1. Allogeneic transplantation is reported in patients with relapsing or refractory disease, but no standard guidelines for its use are available (Perumbeti et al., 2014).

Indications for HSCT in non-malignant diseases

Severe aplastic anemia (SAA)

Allogeneic HSCT is the treatment of choice for patients with SAA who are <40 years of age. However, it must be done soon after onset and before the patients become sensitized by red cell and platelet transfusions (Marsh et al., 2003).

Fanconi's anemia (FA)

FA is an autosomal recessive disease and it is the most common inherited BM failure syndrome. Allogeneic HSCT from an HLA-identical sibling must be considered early in the course of the disease. As patients with FA are prone to DNA damage because of the sensitivity of non-hematopoietic tissues, pre-transplant conditioning with low dose
cyclophosphamide (CY) or fludarabine (FLU) based protocols is used but not irradiation (Alter, 2005).

**β-Thalassemia**

Allogeneic HSCT is the only means of curing β-thalassemia. It should be considered if an HLA-matched sibling donor is available. The risk is low when the transplant is done at an early age (Kumar, 2007).

**Sickle cell anemia (SCA)**

HSCT has a curative potential for SCA. Myeloablative regimens have utilized busulphan (BU) and CY with or without Anti-thymocyte globulin (ATG). The best results are obtained in children who have HLA-identical sibling donors and are transplanted early in the course of the disease (Walter, 2005).

**Stem cell sources (graft sources)**

The HSCs are self-renewable cells, can differentiate to a variety of specialized cells and can mobilize out of the BM into circulating blood. HSCs can be collected from BM, PB, UCB and rarely from fetal liver. Each of these cell sources has specific advantages and disadvantages, and each has found particular applications in the treatment of oncologic or immunologic disorders (Perumbeti et al., 2014). The cellular and clinical characteristics of the commonly used stem cell sources are shown in table (4) (Perumbeti et al., 2014).

**Bone marrow (BM)**

BM is the traditional source of HSCs for use in autologous and allogeneic transplantations. However, the use of PB has replaced BM as a source of these cells for most autologous transplantations and a significant proportion of allogeneic transplantations (Cutler and Antin, 2001). BMT is considered to be superior to PB Stem cell transplantation.
(SCT) for non-malignant conditions (hemoglobinopathies), in which rapid engraftment is not crucial and a graft versus tumor (GVT) effect is not required (Perumbeti et al., 2014).
تنوع في جينات المحركات الخلوية و دورها في التنبؤ بنتيجة زرع النخاع بين الأشقاء المتماثلين

رسالة مقدمة من الطبيب/عبد الله محمد جميل عبدالله

توطئة للحصول على درجة الدكتوراه في الباثولوجيا الاكلينيكية وتحليلاً للأمراض السرطانية

تحت إشراف الأستاذ الدكتور/عزه محمود كامل

استاذ الباثولوجيا الاكلينيكية
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