Evaluation of Cardiac Markers in Children Undergoing Hematopoietic Stem Cell Transplantation

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Background: Early life-threatening cardiotoxicity and cardiac death have been reported after hematopoietic stem cell transplantation (HSCT). The purpose of the current study was to evaluate cardiac toxicity of conventional chemotherapy followed by HSCT with cardiac markers: heart-type fatty acid binding protein (H-FABP), glycogen phosphorylase BB (GPBB), high sensitive C reactive protein (hsCRP) cardiac troponin I, (cTnI), creatine kinase MB (CK-MB mass) and myoglobin. **Methods:** A total of 20 children who underwent HSCT for malignant and non-malignant diseases were included in this study. Blood samples were collected from all patients in 0th, 7th and 21st day for evaluating these cardiac biomarkers. The patients' echocardi-

graphy was assessment before and after one-month of HSCT. **Results:** Serum 21st H-FABP level was significantly higher when compared with the 0th day H-FABP level ($P < 0.05$). 7th day hsCRP level was significantly higher than 0th and 21st day levels ($P < 0.05$). Interestingly, 7th day GPBB level was significantly lower than 0th and 21st day levels ($P < 0.05$). Myoglobin, CK-MB mass and cTnI biomarkers remained within the reference range in all patients. **Conclusions:** This study showed that H-FABP and hsCRP both seem to be promising markers for evaluation of cardiotoxicity in HSCT process and probably superior to GPBB, cTnI, CK-MB mass and myoglobin. J. Clin. Lab. Anal. 29:259–262, 2015. © 2014 Wiley Periodicals, Inc.

Key words: H-FABP; hsCRP; glycogen phosphorylase BB; chemotherapy; children

INTRODUCTION

Hematopoietic stem cell transplantation (HSCT) is the treatment of choice for defined malignant and non-malignant hematological diseases in children (1). HSCT is a complex therapeutic procedure consisting of administration of high-dose chemotherapy, immunosuppressive therapy, and/or radiotherapy, followed by intravenous infusion of hematopoietic stem cells to re-establish marrow function (2). The main drawbacks of HSCT are early transplant-related mortality and delayed complications, which interfere with patient's outcome, health condition, and quality of life. Early life-threatening cardiotoxicity and cardiac death have been showed after HSCT. In comparison with other post-transplant complications, cardiac, or cardiovascular outcomes appear to occur at a much lower frequency (1).

Although there are different techniques to detect cardiotoxicity, research are ongoing for practical, useful, noninvasive, and sensitive methods (3). Cardiac troponins (cTn) and myocardial isoenzyme of creatine kinase MB (CK-MB) are cardiospecific markers that show structural injury of cardiomyocytes from various causes, including cardiotoxic effect of anticancer therapy. Heart-type fatty acid binding protein (H-FABP) and glycogen phosphorylase isoenzyme BB (GPBB) are new markers of myocardial

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ischemia and necrosis recently assessed in the diagnostics and risk stratification of acute coronary syndromes (4). High-sensitivity C reactive protein (hsCRP) also plays a fundamental role in atherosclerosis, ischemic heart failure and coronary heart disease (5).

The purpose of the present study was to evaluate cardiac toxicity of conventional chemotherapy followed by HSCT with cardiac markers of H-FABP, GPBB, hsCRP, cTnI, CK-MB mass, and myoglobin. To our knowledge this is the first study evaluating these cardiac biomarker levels in children who underwent HSCT.

MATERIALS AND METHODS

Patients

A total of 20 consecutive children who underwent HSCT for malignant ($n:12$) and non-malignant diseases ($n:8$) between the ages of 1–20 years at Ankara Children's Hematology and Oncology Hospital. All children included in this study had completed their 100 days after transplantation. Echocardiography was performed for all children before HSCT and 1 month after HSCT.

Written informed consent was obtained from all of the parents and/or subjects. This study was approved by the local ethics committee. The experiments were performed in accordance with the principles enumerated in the Helsinki Declaration of 1975.

Patients characteristic and demographic data of the children have been shown in Table 1.

Hematopoietic Stem Cell Transplantation

Conditioning regimens and GvHD prophylaxis were given according to the current protocols of different diseases. Twenty patients received 11 different conditioning regimens. Most patients (17/20) received myeloablative regimen. ATG at a dosage of 20–40 mg/kg body weight as part of the conditioning regimen was given to four patients. All patients received intravenous immunoglobulin (IVIG) at +1, +8, +15, +22 day, and IVIG was given to all patients whose Ig G level below 400 mg/dL after discharge from the hospital. Cyclosporine A combined with short-term methotrexate was the most preferred protocol (14/20) for acute GvHD. The majority of patients (10/20) received full-matched related HSCT while four patients received autologous HSCT. Only one patient received haploidentical HSCT for Severe Combined Immunodeficiency. Severity of oral mucositis was evaluated and daily graded according to adapted common toxicity criteria for stomatitis in patients (6). The presence and severity of acute GvHD were assessed daily by the attending HSCT physician/nurses and graded according to Glucksberg criteria (7). Steroid was used for

TABLE 1. Patients Characteristic and Biochemical Marker Levels in Study Population

	<i>n</i> (%)		
Diagnosis of patients			
Acute myeloid leukemia	1 (5)		
Acute lymphoblastic leukemia	4 (20)		
Thalassemia Major	2 (10)		
Myelodysplastic syndrome	2 (10)		
Hodgkin Lymphoma	2 (10)		
Primary Hemophagocytic Lymphohistiocytosis	1(5)		
Fanconi aplastic anemia	1 (5)		
Hodgkin Lymphoma	1 (5)		
Hyperimmunoglobulin E syndrome	1 (5)		
Neuroblastoma	1 (5)		
Chediak Higashi syndrome	1 (5)		
Ataxia telangiectasia-mix lineage leukemia	1 (5)		
Wiscott Aldrich syndrome-NHL	1 (5)		
Severe combined immunodeficiency	1 (5)		
Stem cell source			
Bone marrow	14 (70)		
Peripheral stem cell	6 (30)		
Donors			
Sibling	13 (65)		
Father	1 (5)		
Mother	1 (5)		
Uncle	1 (5)		
Autologous	4 (20)		
Donor HLA			
HLA 10/10 compatible	10 (50)		
HLA 9/10 compatible	4 (20)		
HLA 8/10 compatible	1 (5)		
HLA 5/10 compatible	1 (5)		
Autologous	4 (20)		
Conditioning regimen			
Bu-Cy	3 (15)		
TBI-Etoposide	3 (15)		
Flu-Cy-ATG	2(10)		
Pesaro protocol 26	2 (10)		
Bu-Flu-ATG	2 (10)		
Melphalan-Etoposide-Cy	2 (10)		
Melphalan-Etoposide-Carboplatin	2 (10)		
Bu-Cy-Etoposide	1 (5)		
Cy-Melphalan	1 (5)		
Bu-Cy-Melphalan	1 (5)		
TBI-Cy	1 (5)		
Outcome			
Alive without disease	18 (90)		
Died	2 (10)		
	0th day	7th day	21st day
Biomarker			
H-FABP (pg/mL)	588.5 ± 361.9	686.7 ± 326.3	739.5 ± 337.4*
GP-BB (pg/mL)	177.9 ± 68.9	125.7 ± 41.9*	152.7 ± 49.7#
hsCRP (mg/mL)	5.7 ± 5.8	20.1 ± 14.0*	6.5 ± 7.7#

NHL, Non-Hodgkin lymphoma; Bu, Busulphan; Cy, Cyclophosphamide; TBI, Total body irradiation; Flu, Fludarabine.

* $P < 0.05$ in comparison with 0th day levels; # $P < 0.05$ in comparison with 7th day levels.

patients with acute GvHD, chronic GvHD, and engraftment syndrome. Ciprofloxacin, fluconazole, and trimethoprim/sulfamethoxazole were used for antibacterial, antifungal, and *Pneumocystis jiroveci* prophylaxis; respectively. Neutrophil engraftment day was defined as the first of three sequential days in which the absolute neutrophil count was $\geq 500/\text{mm}^3$. Platelet engraftment day was defined as the first of three sequential days in which platelet count was $\geq 20,000/\text{mm}^3$ without transfusion for at least 7 days.

Determination of Biochemical Parameters

After an 8–12 hr overnight fast, the venipuncture was performed between 8:00 am and 9:00 am and blood samples were collected for obtaining serum on 0th day, 7th day, and 21st day after HSCT. Blood samples were centrifuged and serum samples were stored at -70°C until analysis. Measurements of H-FABP, GPBB, and hsCRP were performed in ELISA reader using the commercially available ELISA kit. CK-MB mass, Troponin I, and myoglobin were analyzed using the immunoassay kit. The samples were carried out together in the same experiment.

Statistical Analysis

Data were presented as mean values with standard deviation (SD) for continuous variable. For variables with normal distribution, repeated measures ANOVA was used. Friedman test followed by the Wilcoxon test was used for variables with non-normal distribution. All statistical analyses were performed using the software SPSS 15.0 program. For all statistical tests, two-tailed P value < 0.05 indicated the statistical significance of the results.

RESULTS

Patients and Transplantation Course

A total of 20 children (six girls and 14 boys) with the mean age of 9.5 ± 4.5 years (1–20 years) comprised the study group. Of these 20 children, 16 received allogeneic and four received autologous HSCT. The mean number of CD34 (+) stem cell given to the patients was $4.1 \pm 2.6 \times 10^6/\text{kg}$ ($1.1\text{--}8.7 \times 10^6/\text{kg}$) and the mean number of nucleated cells was $5.6 \pm 2.5 \times 10^8/\text{kg}$ ($2.5\text{--}11.0 \times 10^8/\text{kg}$). Neutrophil engraftment was achieved at median 14 days (11–21) and platelet engraftment, at median 19 days (13–36). Among 20 children who underwent HSCT, 18 had complications related to HSCT after the procedure (15 had febrile neutropenia, 13 had oral mucositis, two had acute GvHD, two had veno-occlusive disease, one had engraftment syndrome, one had hemorrhagic cystitis). The median follow-up duration was 376 days (120–576). The patients' echocardiography was normal before

(Ejection fraction: $74.2 \pm 4.6\%$) and after 1 month of HSCT (Ejection fraction: 70.2 ± 3.8). No patient manifested clinical cardiotoxicity with symptoms of heart failure in the first 100 day after HSCT.

Biochemical Results

In this study, hsCRP levels at 7th day have been significantly increased in comparison with hsCRP levels at 0th day. Moreover, hsCRP level at 21st day has returned to 0th day's levels. If H-FABP levels at 0th and 7th, days were compared; H-FABP levels at 7th day were increased and this increase continued for H-FABP levels at 21st day; whereas only the difference between H-FABP levels at 0th and 21st day was statistically significant. If GPBB levels at 0th, 7th, and 21st days were compared; GPBB levels were decreased at 7th day and those increased at 21st day that reached to baseline level at 0th day. Myoglobin, CK-MB mass, and cTnI biomarkers remained within the reference range in all patients. H-FABP, GPBB, and hsCRP levels at 0th, 7th, and 21st days have been shown in Table 1.

DISCUSSION

Cardiac toxicity is a well known and potentially serious complication during treatment of hemato-oncology patients (8). Thus, we decided to evaluate cardiotoxicity of children who underwent HSCT using cardiotoxicity markers of H-FABP, GP-BB, hsCRP, cTnI, CK-MB mass, and myoglobin.

H-FABP is a 14.5-kDa cytosolic protein present in the myocardium and is a key cytosolic transporter of fatty acids (9). Even in the absence of irreversible myocardial necrosis, H-FABP which is more sensitive and specific than troponin I and CK-MB in the early diagnosis of acute coronary syndrome is released during myocardial ischemia (10). Our findings also, suggest that circulating level of H-FABP is increased at 21st day after HSCT in children who underwent HSCT. It might be associated with myocardial injury manifested by release of H-FABP from cardiomyocytes.

GP-BB is a glycolytic enzyme that plays an essential role in the regulation of carbohydrate metabolism. GPBB isoenzyme is not limited to human heart and brain, and small amounts have been described in numerous other tissues including aorta, leukocytes, testis, digestive tract, spleen, kidney, bladder, and liver (11).

Studies has showed that GP-BB was the most sensitive biomarker to detect myocardial infarction when compared to myoglobin, CK-MB, and cTnT at first hour (12). Horacek et al. found a significant correlation between elevation in GPBB and diastolic LV dysfunction on echocardiography during HSCT (13). Their other study also showed that GPBB seems to be a promising

marker for evaluation of anthracycline-induced cardiotoxicity and probably superior to cTnI and cTnT (14). Interestingly, our results indicate that GPBB levels were decreased at 7th day and those increased at 21st day that was comparable to the level at 0th day. Since, most of our patients received myeloablative conditioning regimen and their leukocyte count was very low at 7th day after HSCT. Furthermore, median neutrophil engraftment day was 14 day after HSCT (11–21 days). Thus, GPBB does not seem to be of value in the detection of cardiotoxicity during HSCT process in children.

A number of prospective research have established that hsCRP is an independent predictor of future risk for cardiovascular events among healthy individuals and among patients with acute coronary syndromes (15). In this study, hsCRP levels at 7th and 21st days have been significantly increased in comparison with hsCRP levels at 0th day.

Horacek et al. evaluated acute and chronic cardiotoxicity of anthracyclines in acute leukemia patients with NT-proBNP, cTnT, and CK-MB mass. They showed that early after anthracyclines administration, even in higher cumulative doses, no patient had a measurable cTnT and increased CK-MB mass concentrations (16). Similarly, our results demonstrated troponin I, CK-MB mass, and myoglobin do not seem to be of value in the detection of early cardiotoxicity in children who underwent HSCT.

In conclusion, this study showed that both H-FABP and hsCRP seem to be promising markers for evaluation of cardiotoxicity in children who underwent HSCT and probably are superior to cTnI, CK-MB mass, myoglobin, and GPBB. Taken all together, these findings could be considered to be a sign of acute subclinical cardiotoxicity. Further studies involving larger numbers of individuals would be valuable to improve our understanding of these cardiac biomarkers in children who underwent HSCT. It is not clear whether these acute changes will have predictive value for development of treatment-related cardiotoxicity in the future. It should be evaluated during prospective follow-up studies.

CONFLICT OF INTEREST

The authors report no conflict of interest.

REFERENCES

1. Tichelli A, Bhatia S, Socie G. Cardiac and cardiovascular consequences after haematopoietic stem cell transplantation. *Br J Haematol* 2008;142(1):11–26.
2. Tavit B, Koksall E, Yalcin SS, Uckan D. Pretransplant nutritional habits and clinical outcome in children undergoing hematopoietic stem cell transplant. *Exp Clin Transplant* 2012;10(1):55–61.
3. Kismet E, Varan A, Ayabakan C, Alehan D, Portakal O, Buyukpamukcu M. Serum troponin T levels and echocardiographic evaluation in children treated with doxorubicin. *Pediatr Blood Cancer* 2004;42(3):220–224.
4. Horacek JM, Vasatova M, Tichy M, Pudil R, Jebavy L, Maly J. The use of cardiac biomarkers in detection of cardiotoxicity associated with conventional and high-dose chemotherapy for acute leukemia. *Exp Oncol* 2010;32(2):97–99.
5. Masugata H, Senda S, Inukai M, et al. Association between high-sensitivity C-reactive protein and left ventricular diastolic function assessed by echocardiography in patients with cardiovascular risk factors. *Tohoku J Exp Med* 2011;223(4):263–268.
6. National Cancer Institute CTEP. Common toxicity criteria v2.0. Available at: <http://ctep.cancer.gov/reporting/CTC-3test.html>. Accessed on March 15, 2013.
7. Rowlings PA, Przepiorka D, Klein JP, et al. IBMTR Severity Index for grading acute graft-versus-host disease: Retrospective comparison with Glucksberg grade. *Br J Haematol* 1997;97(4):855–864.
8. Pudil R, Vasatova M, Lenco J, et al. Plasma glycogen phosphorylase BB is associated with pulmonary artery wedge pressure and left ventricle mass index in patients with hypertrophic cardiomyopathy. *Clin Chem Lab Med* 2010;48(8):1193–1195.
9. Burton PB, Hogben CE, Joannou CL, et al. Heart fatty acid binding protein is a novel regulator of cardiac myocyte hypertrophy. *Biochem Biophys Res Commun* 1994;205(3):1822–1828.
10. Orak M, Ustundag M, Guloglu C, Ozhasenekler A, Alyan O, Kale E. The role of the heart-type fatty acid binding protein in the early diagnosis of acute coronary syndrome and its comparison with troponin I and creatine kinase-MB isoform. *Am J Emerg Med* 2010;28(8):891–896.
11. Apple FS, Wu AH, Mair J, et al. Future biomarkers for detection of ischemia and risk stratification in acute coronary syndrome. *Clin Chem* 2005;51(5):810–824.
12. Rabitzsch G, Mair J, Lechleitner P, et al. Isoenzyme BB of glycogen phosphorylase b and myocardial infarction. *Lancet* 1993;341(8851):1032–1033.
13. Horacek JM, Jebavy L, Ulrychova M, et al. Glycogen phosphorylase BB could be a new biomarker for detection of cardiac toxicity during hematopoietic cell transplantation for hematological malignancies. *Bone Marrow Transplant* 2010;45(6):1123–1124.
14. Horacek JM, Tichy M, Jebavy L, Pudil R, Ulrychova MJM. Use of multiple biomarkers for evaluation of anthracycline-induced cardiotoxicity in patients with acute myeloid leukemia. *Exp Oncol* 2008;30(2):157–159.
15. Goud BK, Nayal B, Devi OS, et al. Comparison of microalbuminuria with hs-CRP and low density lipoprotein levels in nondiabetic, nonhypertensive myocardial infarction patients. *J Cardiovasc Dis Res* 2012;3(4):287–289.
16. Horacek JM, Pudil R, Jebavy L, Tichy M, Zak P, Maly J. Assessment of anthracycline-induced cardiotoxicity with biochemical markers. *Exp Oncol* 2007;29(4):309–313.