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The Relationship between the Lifestyle of the Allogeneic Stem Cell Donors and the Number of Donated $CD34^+$ and $CD3^+$ Cells

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ABSTRACT
Background: Hematopoietic stem cell transplantation (HSCT) is considered as the last treatment option in many life-threatening diseases. The number of donated cells can affect transplantation success. This study attempted to investigate the relationship between the health-promoting lifestyle of allogeneic stem cell donors and the number of donated CD34+ and CD3+ cells.
Materials and Methods: The study was a descriptive correlational study in which 100 peripheral blood stem cells donors participated. A demographic form and health-promoting lifestyle profile-II questionnaire were distributed to participants, and then cell separation was started. Afterward, the results of CD34 + and CD3 + cell counts, as well as other clinical parameters of the participants, were recorded. The collected data were analyzed by descriptive and analytical statistical methods.
Results: The results showed that the mean total health-promoting lifestyle profile score for hematopoietic stem cell donors was 2.876±0.461. There was no significant relationship between the health- promoting lifestyle score and the number of CD34+, CD3+ cells and CD3+/CD34+ ratio. A positive and significant correlation was found between the weight of the donors and the number of CD34+ ($P < 0.001$) and CD3+ cells ($P = 0.001$). The number of CD34+ cells was significantly different between women and men ($P = 0.009$).
Conclusion: Lifestyle had no significant effect on the number of CD3+/CD34+ cells. Moreover, the number of CD34+ cells was significantly higher in men, so males should be preferentially recruited as donors for the HSCT procedure.

Keywords: Hematopoietic stem cell transplantation; Stem cell, Collection; Lifestyle; Donor

INTRODUCTION

Over the past decades, the rate of hematopoietic stem cell transplantation (HSCT) has exceeded 60,000 per year worldwide[1]. Allogeneic HSCT, in which hematopoietic stem cells (HSCs) are obtained from a donor and injected into the recipient, is widely used in the treatment of hematologic disorders. Currently, peripheral blood stem cells (PBSCs) separating through apheresis are used as one of the primary sources for allogeneic transplants. Apheresis is defined as the process of removing a specific component of blood and returning the rest of the blood to the donor. The type of apheresis depends on the specific component removed. Apheresis is used for both therapeutic and donation purposes[2,3].

Stem cells express CD34$^+$ marker. A low number of CD34$^+$ cells obtained from the donor during apheresis or insufficient function of these cells are associated with graft failure in HSCT. Besides CD34$^+$ cells, B and T lymphocytes are also present among the isolated cells. CD3$^+$ markers are indicators of T lymphocytes. On one hand, the higher the number of CD34$^+$ cells, the higher the probability of transplant success[4]. On the other hand, the higher the number of T lymphocytes, the higher the rate of graft rejection and graft-versus-host disease (GVHD)[5]. HSCT failure is followed by the need for re-transplantation, re-treatment, incremental costs, and an even higher risk of death. Donor's conditions affect the number and quality of CD34+ cells. For example, a study showed that CD34+ cells in donors ≥ 60 years old with underlying diseases, such as blood pressure and heart condition, had inferior performance compared to the young people[6]. Another study showed that smoking is the main factor that affects the donor's number of peripheral blood cells and cell function[7]. Donor's anxiety level also affects the number of CD34+ and CD3+ cells[8]. However, the results of studies are contradictory, and further studies are suggested. For instance, age was considered a factor that affects the function of CD34+ after cell collection[9], but other studies concluded otherwise[10].

Several recommendations have been made regarding the evaluation of the health of HSCs donors to protect recipients of hematopoietic stem cell transplantation and apheresis. These recommendations include examination of infectious diseases, heart disease, allergies, diabetes, cancer, and other factors, such as mental illnesses, depression, health conditions at the time of donation, weight, height, tobacco, alcohol, and drug use[10,11]. However, some studies have shown that lifestyle affects blood composition, and the measurement of blood compounds reveals the amount of a person's physical activity and what they have eaten or drunk[12] . The developed health models have also mentioned the role of lifestyle and background factors, such as social, economic, and environmental conditions, as well as individual characteristics on blood products[13,14]. The concept of a healthy lifestyle was proposed by Pender. From this perspective, lifestyle includes behaviors controlled by a person and affect a person's health behaviors[15]. The lifestyle based on Pender's health promotion model emphasizes various dimensions of nutrition, physical activity, health responsibility, stress management, interpersonal relationships, and spiritual growth[16].

Because HSCT is a relatively new science, there are few studies that have examined the effects of donor demographic, clinical, or lifestyle characteristics on stem cell function and CD34+ and CD3+ cell counts. Some studies have shown that a sedentary lifestyle, unhealthy diet, heavy alcohol consumption, and smoking negatively affect hematopoietic stem cell niches[17]. Moreover, diet choices, sleeping patterns, physical exercise, and psychosocial stress can influence stem cells[18]. Restriction of proteins and amino acids in the diet may also negatively affect stem cell function[19]. Overall, it appears that lifestyle factors may affect stem cell function, but more research is needed verify this assumption. We aimed to determine the relationship between the lifestyle of HSC donors and the number of donated CD34+, CD3+, and CD3+/CD34+ cells.

MATERIALS AND METHODS

This investigation was a descriptive correlational study that was conducted between March 2021 and March 2022.

Setting and participants

This study was conducted in Shariati Hospital, one of the main HSCT centers in the country, equipped with a cell separation department that, on average, performs 40 cases of cell collection per month. Following the doctor's order, a G-SCF injection was performed. On the day of cell collection, donors went to the cell separation unit and were connected to the apheresis machine for cell collection. The process usually took 4 to 8 hours, depending on factors like the recipient's weight and the quality of the donated cells. Peripheral blood cells were sent to the flow cytometry laboratory for the measurement of CD3$^+$ T cells and CD34$^+$ stem cells. When the physician confirmed the injection, the bag containing the stem cells was sent to the transplant department. The sample of this study included 100 HSC donors referred to the apheresis department of Shariati Hospital. The inclusion criteria were: age 18-65 years, *availability* of an *HLA-matched donor* , doctors' approval to donate HSC, apheresis capability (separation of HSC from peripheral blood for allogeneic transplantation) in the cell separation unit of the hospital, , understanding and speaking in Persian language, ability to read and write, and written consent to participate in the study.

Sample size

Due to the lack of study on the relationship between the lifestyle of HSC donors and the number of $CD34^+$ or $CD3^+$ counts, to determine the sample size, basic information, including the regression coefficient of the relationship between the Beck Anxiety Inventory (BAI) score and $CD3^+$ count was obtained from Larijani et al.'s study[8]. PASS15 software was used to calculate the minimum required sample size (100 cases) at a 95% confidence intervaland 90% test power. The participants were selected by the available sampling method, and sampling continued until reaching the sample size of 100.

Data collection tools

Relevant data were collected using a researcher-made questionnaire for demographic information and the donors' clinical variables, a cell count registration form, and the health-promoting lifestyle profile II (HPLP II).

Demographic information and clinical variables of the participants were gathered regarding age, gender, height, weight, marital status, occupation, relationship with the recipient, type of recipient's disease, the amount of received granulocyte colony-stimulating factor (G-CSF), and the duration of cell separation. HPLP II questionnaire was developed based on the framework of Pender's Health Promotion Model[20]. The questionnaire includes 52 items, and participants choose a response on a four-point scale by indicating never/sometimes/often/usually (scored 1 to 4, respectively). The 52 items are divided into six dimensions: nutrition (9 questions), physical activity (8 questions), health responsibility (9 questions), stress management (8 questions), interpersonal relationships (9 questions), and spiritual growth (9 questions). The overall score of the questionnaire was calculated by adding all the scores of all the questions and dividing the sum by the total number of questions. The mean overall score of the questionnaire is between 1 and 4, and the mean score in each of the above-mentioned dimensions is also between 1 and 4[16]. The questionnaire had already been translated into Persian and used in Iran[16,21]. Previous studies reported acceptable reliability for the questionnaire[21,22]. The mentioned questionnaires were approved by ten faculty members of the School of Nursing and Midwifery as well as the Hematologists of the Research Center, and then validity and reliability were calculated. The calculated reliability of the HPLP II using Cronbach's alpha was 78%.

Other calculated parameters included $CD34^+$ and $CD3^+$ cell counts and blood parameters, such as white blood cell (WBC) and red blood cell (RBC) count, hemoglobin(Hb), hematocrit(HCT), and platelet (Plt) count from patient records, cell separation time in minutes, total blood volume (TBV) in ml, and total process blood volume (TPBV) in ml. Stem cell collected volume in ml was obtained from the cell separation unit, and CD3+/CD34+ ratio, another indicator of PBHSCs apheresis success, was also calculated.

Data collection

In the separation unit, apheresis procedures were explained to volunteer donors before G-CSF injection.

On the day of cell separation, inclusion criteria were controlled. Informed *written consent* was *obtained* from all participants prior to participation in the study, and then they were asked to complete the demographic and HPLP II questionnaire in a quiet room.

Afterward, the donors were connected to the apheresis machine. After the completion of cell collection, the cells and blood samples were sent for flow cytometric analysis, which was conducted by the experts and hematologists of the Hematology, Oncology and Stem Cell Transplantation Research Center. The laboratory calculated the number of cells

(WBC Count), which was then multiplied by 1000, multiplied by the TPBV, and finally divided by the weight of the recipient (the resulting number was considered the reference number). In addition, the flow cytometer was used to determine the percentage of CD3+ and CD34+ in the TPBV apheresis product. To determine the number of CD3+ per Kg (CD3+/kg) and CD34+/kg, the indicated percentage was multiplied by the obtained number (the reference number). Moreover, the total number of CD3+ and $CD34^+$ was calculated by multiplying $CD3^+$ /kg and $CD34^+$/kg by the patient's body weight. It should be noted that in allogeneic PBSCT, the optimal number of $CD34^+$ cells for HSCT has not been definitively determined, but a dose higher than 5×10^6 $CD34^+$ cells per kilogram of body weight is preferred, and the same was considered in the present study.

It is worth mentioning that the weight of the donors and recipients was measured on a standardized scale. The duration of separation was also measured by the time that recorded by the machine. All other laboratory parameters were accurately measured by experts.

Data analysis

Data were analyzed using SPSS software (version 16). Qualitative variables are summarized and reported as frequency and percentage. Quantitative variables are reported as mean (standard deviation). Considering the normality of the data, Pearson, independent samples t-test and univariate analysis were used to check the relationship between the variables. The significance level was set at $P < 0.05$.

RESULTS

The average age of HSC donors was 38.79±11.733 years. Most of the donors (61%) were men. The donors' average height was 170.61±8.949 cm, and the average body mass index (BMI) was 26.908 ± 5.154 Kg/m^2. Other demographic characteristics of donors are shown in Table 1.

The average HPLPII score of the donors was 2.876±0.461. The average scores for physical activity, stress management, health responsibility, nutrition, spiritual growth, and interpersonal relationships were 2.55±0.678, 2.70±0.562, 2.99±0.496, 2.79±0.472, 3.07±0.563, and 3.07±0.482, respectively. The mean number of $CD34^+$ cells of the donors was $12.481 \pm 7.392 \times 10^6$ /kg, and the mean number of $CD3^+$ cells was $412.18 \pm 202.937 \times 10^6$ /kg. The mean TBV was 4838.180±1008.532 ml (Table 2). Results showed no significant relationship between the total HPLP II score or HPLP II dimensions and the number of $CD34^+$ or $CD3^+$ cells. A positive and significant correlation was found between the weight of the donors and the number of $CD34^+$ cells ($P < 0.001$) and the number of $CD3^+$ cells ($P = 0.001$), which indicated an increase in the number of cells with the increase in weight. A significant negative correlation was found for $CD3^+/CD34^+$ ratio ($P = 0.01$). The correlations between the number of WBCs and the number of $CD3^+$ cells ($P = 0.038$), between the HCT and the number of $CD34^+$ cells ($P = 0.035$), between TBV and $CD34^+$ ($P < 0.001$) and $CD3^+$ count ($P = 0.05$), and between TPBV and $CD34^+$ ($P = 0.011$) and $CD3^+$ count ($P < 0.001$) were all positive and significant. The number of $CD34^+$ cells was significantly different between men and women ($P = 0.009$), and the average number of $CD34^+$ cells was higher in men. Moreover, the ratio of $CD3^+$/ $CD34^+$ was significantly different between men and women ($P=0.003$); therefore, the ratio was higher in women (Table 3).

Table 1: Demographic and clinical characteristics of hematopoietic cell donors

		Mean ± SD / N (%)
Age(year)		38.79±11.733
Age (year)	18-30 (year)	24(24.0)
	30-45 (year)	48(48.0)
	45-65 (year)	28(28.0)
Gender	Male	61(61.0)
	Female	39(39.0)
Marriage status	Single	26(26.0)
	Married	74(74.0)
Job	Employee	33(22.0)
	Freelance	29(29.0)
	Housewife	22(22.0)
	Retired	6(6.0)
	Student	10(10.0)
Relationship with the recipient	Brother or sister	69(69.0)
	Father or mother	18(18)
	Child	2(2,0)
	Second- or third-born sibling	4(4.0)
	Not a relative	7(7.0)
Height (Cm)		170.61±8.949
Weight (Kg)		78.51±17.046
Weight by gender	Male	83.34±16.208
	Female	70.97±15.70
Body mass index (Kg/m^2)		26.908 ± 5.154
Body mass index	< 18.5 (Underweight)	4(4.0)
	18.5–24.9 (Normal weight)	33(33.0)
	25.0–29.9 (Overweight)	36(36.0)
	30.0–34.9 (Class I obesity)	22(22.0)
	35.0–39.9 (Class II obesity)	4(4.0)
	≥ 40 (Class III obesity)	1(1.0)
Cell separation duration (minutes)		245.73±75.281
G-CSF (µg/kg/day)		10.596±2.374
WBC×10^3/ml		55.70±14.897
RBC×10^6		5.19±3.625
Hb (g/dl)		14.57±1.734
HCT (%)		40.08±4.553
Platelet ×10^3/ml		206.54±51.329
Donor recipient body weight ratio (D/R ratio)		1.679±1.427

Table 2: The mean scores of HPLPII, CD34+, and CD3+ counts of HSCT donors

Variables		Mean ± SD /Median	Min-Max / (IQR)*
HPLP II	Total	2.876±0.461	1.90-3.9
	Physical activity	2.55±0.678	1.00–4.00
	Stress management	2.70±0.562	1.75–4.00
	Health responsibility	2.99±0.496	2.00–4.00
	Nutrition	2.79±0.472	1.56–3.78
	Spiritual growth	3.07±0.563	1.78–4.00
	Interpersonal relationships	3.07±0.482	1.78–4.00
$CD34^+ \times 10^6$ /kg		12.481±7.392/10.49	3-58.70/5.727
$CD34^+ \times 10^6$ (Total)		758.754±474.312/675.59	92.46-2689.20/662.567
$CD3^+ \times 10^6$ /kg		412.18±202.937/370.50	124-1690/125.00
$CD3^+ \times 10^6$ (Total)		23979.240±10914.90/23200	1723.12- 64746.0/11507.25
TBV/ Donor (ml)		4838.180±1008.532/4833.00	3001.0-8184.0/1348.75
TPBV (ml)		12689.06±4430.297/12355.00	1209.0-25850.0/5985.5
Stem Cell Collected Volume (ml)		324.939±93.777/301	141.0-580.0/133

*Inter Quartile Range: The difference between the 75th and 25th percentiles of the data

Table 3: The results of the univariate analysis to evaluate the relationship between HPLP II and participants' characteristics with CD34+, CD3+ cell count, and CD3+/CD34+ ratio

Variables		CD34+ Pearson Correlation Coefficient (r)	CD34+ *P* value	CD3+ Pearson Correlation Coefficient (r)	CD3+ *P* value	CD3+/ CD34+ Pearson Correlation Coefficient (r)	CD3+/ CD34+ *P*
HPLP II	Total Score	-0.042	0.676	-0.142	0.159	-0.051	0.616
HPLP II	Physical Activity	-0.149	0.139	-0.211	0.035	-0.011	0.910
HPLP II	Stress management	-0.056	0.582	-0.175	0.082	-0.089	0.380
HPLP II	Health responsibility	0.043	0.672	-0.02	0.845	-0.035	0.731
HPLP II	Nutrition	-0.015	0.878	-0.122	0.226	-0.036	0.720
HPLP II	Spiritual growth	0.005	0.96	-0.097	0.338	-0.061	0.546
HPLP II	Interpersonal relationships	-0.024	0.811	-0.086	0.394	-0.032	0.755
Age (Years)		0.075	0.46	0.1	0.324	-0.153	0.129
Duration of separation (Minute)		0.041	0.687	0.268	0.007	0.109	0.282
Hight (Meter)		0.208	0.038	0.095	0.345	-0.138	0.171
Weight (Kg)		0.356	<0.001	0.339	0.001	-0.257	0.010
D/R ratio		-0.411	<0.001	-0.514	<0.001	0.018	0.859
G-CSF (µg/kg/day)		-0.115	0.253	-0.013	0.901	0.090	0.375
WBC		0.188	0.061	0.208	0.038	-0.263	0.008
Hb(mg/dl)		0.187	0.063	-0.125	0.214	-0.292	0.003
HCT (%)		0.211	0.035	-0.083	0.41	-0.268	0.007
PLT		0.028	0.783	0.163	0.106	0.017	0.870
TBV/ Donor		0.348	<0.001	0.196	0.05	-0.343	<0.001
TPBV		0.254	0.011	0.387	<0.001	0.034	0.736
Stem Cell Collected Volume		0.297	0.003	0.515	0.515	0.034	0.741
Gender	Male (Mean±SD)	849.30±525.20	0.009*	24080.86±11834.60	0.908*	34.1113±16.357	0.003*
Gender	Female(Mean±SD)	617.13±341.81		23820.30±9444.16		46.304±23.580	

Degree of significance of P i: P <0.05

* Independent Samples t-Test

DISCUSSION

The results showed that the average age of HSCT donors participating in the study was 38.79±11.733 years, of whom 61% were men, which is consistent with previous studies[8,23-25]. The mean duration of HSCT separation was 245.73±75.281 minutes. On average, the donors received 10.596±2.374 G-CSF (µg/kg/day). The mean of white blood cells, Hb, HCT, and Plt count of donors after G-CSF injection was 55.70±14.897, 14.57±1.734, 40.08±4.553, and 206.54±51.329, respectively. The results of a study conducted in Iran regarding the effect of anxiety state on the number of CD3+ and CD34+ cells also showed that after the injection of 8.04±2.45 G-CSF (µg/kg/day) the mean of the WBCs, Hb, HCT, and Plt count was 44.45±11.20, 14.15±1.62, 42.64±3.78, and 231.5±57.5, respectively[8]. The mean of CD34+ cell count of the HSC donors was 12.481±7.392×10^6 /kg, and the mean of the number of CD3+ cell count was 412.18±202.937 ×10^6 /kg. In a study conducted in Iran, the mean of CD34+ and CD3+ cells were 58.90±47.14 and 89.336±104.64 respectively[8].

The mean scores and HPLP II of HSC donors and its' dimensions showed that the dimensions of spiritual growth, interpersonal relationships, and health responsibility had the highest scores (3.07±0.563, 3.07±0.482, and 2.99±0.496, respectively), followed by nutrition, stress management, and physical activity (2.79±0.472, 2.70±0.562, and 2.55±0.678, respectively). The mean score of the total lifestyle of HSC donors was 2.876±0.461, indicating an average lifestyle. No significant correlation was found between the number of cells and any dimensions of the HPLP II or the total HPLP II score. In a study conducted in Iran to investigate the effect of anxiety on the number of CD34+ cells, 111 donors entered the study. The mean score of anxiety was found to be 22.85±15.43 (mild to medium) among study participants, indicating a significant relationship between anxiety and the number of CD34+ cells. In addition, the anxiety score had a significant impact on the number of CD3+ cells[8] Another study also reported the effect of panic disorder on the number of PBHSCs[26]. However, this study showed that there was no significant relationship between the stress management score and the number of cells. A study conducted in the US (2022) reported no relationship between a donor's health-related quality of life (HRQoL) and the number of cells[27].

The difference between the number of CD34+ cells in men and women was statistically significant. Like previous studies, the mean number of CD34+ cells was higher in men[25]. The results also showed a significant positive correlation between the donors' weight and the number of CD34+ and CD3+ cells. Studies have pointed to the effect of BMI on cells[25]. There was a significant positive correlation between the number of WBCs and the number of CD3+ cells-, between HCT and the number of CD34+ cells, between TBV and both the number of CD34+ and CD3+ cells, and between TPBV and the number of CD34+ and CD3+ cells. Other studies reported that factors such as age, gender, number of WBCs, Plt, and precollection HPC were effective in successful allogeneic PBSC collection in healthy donors[25,28].

Since the present study could not show a relationship between lifestyle and the number of cells, multicenter studies with sufficiently large sample sizes and using other methods of lifestyle assessment may determine the relationship or lack of a relationship between lifestyle and the number of CD34+ and CD3+ cells.

Limitations

This research employed a non-probability sampling design using a standardized self-report questionnaire to collect lifestyle data, which cannot be considered a suitable replacement for an objective lifestyle assessment.

CONCLUSION

The findings showed no significant relationship between the number of $CD3^+$ or $CD34^+$ cells and HPLP II or its dimensions, and lifestyle had no significant impacts on the number of these cells. Moreover, the level of CD34 cells was higher in men, so males should be preferentially recruited as donors for the HSCT procedure.

Ethical Consideration

This study was approved by the research ethics committees of the schools of nursing & midwifery and rehabilitation (IR.TUMS.FNM.REC.1400.054) - Tehran University of Medical Sciences in 2021. Informed consent was obtained from all participants.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this paper.

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