Original Research Article

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T cell receptor excision circles (TRECs) and kappa-deleting recombination excision circles (KRECs) as markers of early T cell and B cell immune recovery after haematopoietic stem cell transplantation (HSCT)

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Abstract

Hematopoietic stem cell transplantation (HSCT) is a key therapeutic strategy for a number of hematological malignancies and non-malignant disorders of the hematopoietic system. One of the most important events post SCT is the immune system reconstitution—a process characterized by a considerable dynamic and is critical in promoting overall survival of transplant patients, restoring immune protection from opportunistic infections, and mediating an alloreactive graft-versus-tumor effect against residual malignant disease. T cell receptor excision circles (TRECs) and Kappa deleting recombination excision circles (KRECs) are byproducts of T cells and B cells receptor recombination processes, respectively and can be used in monitoring denovo synthesis of T cells and B cells post SCT. The aim of this study was to determine the role of combined TRECs and KRECs quantitation in follow up of allogenic HSCT patients through testing samples at 1, 3 and 6 months post-transplant as well as their role in predicting outcomes of transplantation. The study was conducted on 32 patients receiving allogenic HSCT from an HLA identical sibling. Combined quantification of TRECs and KRECs in the genomic DNA of peripheral blood mononuclear cells was performed using quantitative real-time PCR using a standard curve. TRECs and KRECs levels were inversely related to age and significantly lower in patients transplanted for malignant diseases than benign diseases ($p < 0.05$). TREC levels could predict relapse as an outcome and graft versus host disease (GvHD) at 6 months posttransplant. In conclusion, age and nature of disease determined TRECs and KRECs levels posttransplant. In addition, monitoring immune reconstitution using combined TRECs/KRECs quantitiation by real time PCR may be informative and could help predict outcomes of transplantation in allogenic HSCT.

Keywords: HSCT, TRECs, KRECs, Monitoring immune reconstitution, thymic function, Simultaneous TREC/KREC quantification assay.

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Introduction

Hematopoietic stem cell transplantation nowadays is a widely accepted, potentially curative treatment strategy for patients with refractory hematological malignancies and a variety of benign disorders. Despite substantial advances in HLA matching and transplant procedures, mortality and morbidity following hematopoietic stem cell transplantation (HSCT) remain a problem, in large part due to graft versus host disease (GvHD) stemming from alloreactivity and variable immune reconstitution contributing to infections.[1]

A timely and balanced T cell and B cell reconstitution following HSCT is a crucial factor in determining clinical outcomes. Though progress has been made for better understanding of this process, the complexities inherent to immune recovery after HSCT are still elusive.[2] This is particularly the case for the T cell pool, which not only mediates GvHD and protection against opportunistic infections but is also crucial to the graft versus leukemia effect (GvL) of an allograft[1]. Reconstitution of the donor-derived immune system is essential for modulation of GvHD, and relapse control, thus contributing to long-term survival.[3] New functional measures of immune recovery and chimerism would, therefore, guide clinicians in the assessment of engraftment and immune response beyond simple absolute lymphocyte counts. Immune monitoring is of major importance to identify patients at risk and provide opportunities for early immune interventions. For instance, patients with low T-cell counts early after HSCT are at risk for HSCT-related complications and could benefit from adjuvant immunotherapies, such as low-dose IL-2, administration of T regs, or leukemia/virus specific dendritic cells (DCs) and/or specific T cells.[4]

Reconstitution of adaptive immunity requires continuous generation of new T and B cells, that are able to maintain their proliferative capacity. This entails active contribution of both the thymus and bone marrow (BM) in terms of thymopoiesis and B-cell neogenesis, respectively.[5] During T and B cell development, premature T and B cells undergo the process of recombination of receptor gene segments. This occurs at the T cell receptor (TCR) and B cell receptor (BCR) loci to generate the diversity of T and B cell repertoires, respectively. These recombination processes result in the formation of extrachromosomal non-replicating excision circles namely T cell receptor excision circles (TRECs) and Kappa deleting recombination excision circles (KRECs).[6] TRECs are generated during the latest stages of T cell development just before they exit to the periphery. Hence, their levels in the peripheral circulation represent thymic activity[7] and represents a reliable marker for thymic output. Conversely, KRECs are formed in the early stages of development up to mature B lymphocytes.[5] Both TRECs and KRECs are stable and non-replicative and are subsequently diluted during cell proliferation. Hence, TRECs and KRECs analyses have been widely performed in different clinical settings to evaluate thymic and bone marrow output.[8]

The follow up of patients following HSCT is a major challenge and a burden to health care. Monitoring thymic output in HSCT patients have significantly improved our understanding of main issues related to HSCT and has allowed researchers to identify factors affecting thymic recovery post-HSCT. Thymic function is believed to be a reliable predictor for morbidity and mortality post HSCT.[9] The challenge for the next decades will be to translate advances in immunemonitoring into a better donor selection and how to identify the proper recipient.[10] Combined TRECs and KRECs analysis will allow the implementation of a new tool for immune monitoring to help in proper management of HSCT patients attending Ain Shams HSCT Center from all over Egypt according to high standards for the sake of better survival rates, outcomes, quality of life, and better money investment. Therefore, the aim of this study was to determine the role of combined TRECs and KRECs quantitation in follow up of allogenic HSCT patients through testing samples at 1, 3 and 6 months post-transplant as well as their role in predicting outcomes of transplantation.

Subjects and Methods

This prospective cohort study was conducted at Ain Shams University Hospitals and included 32 patients who had undergone allogenic HSCT for a variety of malignant and non-malignant diseases. The study protocol was reviewed and approved by the Research Ethics Committee, Faculty of Medicine, Ain Shams University (FMASU MD 174/ 2018). A written informed consent was taken from all the patients before participation in this study.

For quantitative real time PCR of TRECs and KRECs five mL of venous blood were drawn using EDTA vacutainer tubes from every patient at 1-, 3- and 6-months post -transplant. Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll-Hypaque (Lymphoprep, Axis-Shield, Scotland, UK) density gradient centrifugation. Then, PBMCs were frozen at -20C for subsequent DNA extraction.

DNA was extracted using the QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA), according to the manufacturer's instructions. The number of TRECs and KRECs was simultaneously determined by a duplex Real-Time quantitative polymerase chain reaction (qPCR) assay performed using the Rotor Gene Q MDx (QIAGEN, Hilden, Germany). TRECs forward primer and probe were those designed by Douek et al., 1998.[11] The reverse primer for TRECs, together with primers and probe for KRECs and primers and probe for T Cell Receptor Alpha Constant (TRAC) gene (reference gene) were those designed by Sottini et al., 2014.[12] (Table 1)

PCR reactions were developed in a final volume of a 25-µl mixture, consisting of 5 µl (400-500 ng) of genomic DNA solution, 12.5 µl of 2× TaqMan Universal PCR master mix consisting of 1x Buffer, 1.5 mM $MgCl_2$, dNTP mix (200 µM each), Amplitaq DNA polymerase (2.5U/25µl), and primers and probes for TRECs and KRECs at the final concentration of 900 nM and 200 nM, respectively (1.125µl each). Amplification of the TRAC reference gene was done in the same plate with the same concentrations of TRAC- specific primers and probe. The assay was run for the three genes with the same standard protocol consisting of an initial heating at 95 °C for 10 min, followed by 45 cycles, each of denaturation at 95 °C for 15 seconds and a combined primer/probe annealing and elongation at 60 °C for 1 min. TRECs, KRECs and TRAC numbers were obtained by extrapolating the respective sample quantities from the standard curve obtained by serial dilutions (10^6,10^5,10^4,10^3,10^2 and 10) of the linearized triple-insert plasmid DNA which were amplified in each PCR plate. A standard curve was included in every PCR run by serial dilution of a triple insert plasmid DNA that was kindly supplied by Dr Luisa Imberti and Dr Alessandra Sottini Laboratorio Crea, Spedali Civili Di Brescia, Italy. The number of TRECs or KRECs was calculated per million of cells.

Statistical analysis

Data were gathered, edited, coded, and entered in a computer using IBM SPSS version 23 (Statistical Package for Social Science) (Armonk, NY). The quantitative data were presented as mean, standard deviations and ranges when their distribution was found parametric and median with inter-quartile range (IQR) when their distribution was found non-parametric. Also, qualitative variables were presented as number and percentages. The comparison between groups with qualitative data was done by using Chi-square test. The comparison between two groups with quantitative data and parametric distribution were done by using independent t-test while the comparison between two groups with quantitative data and nonparametric distribution was done by using Mann-Whitney test. Spearman correlation coefficients were used to assess the correlation between two quantitative parameters in the same group. The confidence interval was set to 95% and the margin of error accepted was set to 5%. A p value of < 0.05 was considered significant.

Table 1. Sequence of primer and probes for the real-time PCR assay.

TRECs	forward	5’-CAC ATC CCT TTC AAC CAT GCT-3’
	reverse	5’-TGC AGG TGC CTA TGC ATC A-3’
	probe	5’-FAM-ACA CCT CTG GTT TTT GTA AAG GTG CCC ACT-TAMRA-3’
KRECs	forward	5’-TCC CTT AGT GGC ATT ATT TGT ATC ACT-3’
	reverse	5’-AGG AGC CAG CTC TTA CCC TAG AGT-3’
	probe	5’-HEX-TCT GCA CGG GCA GCA GGT TGG- TAMRA-3’
TCRAC	forward	5’-TGG CCT AAC CCT GAT CCT CTT-3’
	reverse	5’-GGA TTT AGA GTC TCT CAG CTG GTA CAC-3’
	probe	5’-FAM-TCC CAC AGA TAT CCA GAA CCC TGA CCC-TAMRA-3’

Results

Of the 32 patients included in the study, 13 (40.6%) were females and 19 (59.4%) were males. Their age ranged from 1.5 to 41 years. Eleven cases (34.4%) were diagnosed with benign diseases and 21 cases (65.6%) were diagnosed with malignant diseases. Table [2]

Table 2. Demographic data and diagnosis of the 32 studied patients.

Studied parameter	Item	Value
Gender	Females	13 (40.6%)
	Males	19 (59.4%)
Age (years)	Median (IQR)	21 (11.5 – 31.5)
	Range	1.5 – 41
Nature of disease	Benign	11 (34.4%)
	Malignant	21 (65.6%)
Diagnosis	Acute myeloid leukemia (AML)	12 (37.5%)
	Acute lymphocytic leukemia (ALL)	8 (25.0%)
	Beta thalassemia	4 (12.5%)
	Aplastic anemia	3 (9.4%)
	Fanconi	2 (6.2%)
	Osteoporosis	1 (3.1%)
	Hypoplastic myelodysplastic syndrome (MDS)	1 (3.1%)
	Familial hemophagocytic lymphohistocytosis (HLH)	1 (3.1%)

On follow-up of HSCT patients, a significant statistical difference was observed in both TRECs/ million of cells and KRECs/ million of cells, with an increase from 1 month to 3 months and to 6 months post-transplant ($P < 0.0001$) (Table 3, Figures 1 and 2). Regarding outcomes of transplantation, of the 32 patients included in the study, relapse occurred in 4 patients, GvHD occurred in 13 patients and 9 patients died due to transplant related causes within the 1 year follow up period (Table 4). There was a negative correlation between patients’ age in years and TRECs and KRECs measured at 1, 3 and 6 months post-HSCT (Table 5).

Table 3. Follow up of TRECs/million of cells and KRECs/million of cells at different times of measurement post-transplant [1 month, 3 months and 6 months] in the 32 studied subjects.

		1 month	3 months	6 months	≠*P* value
TRECs/million of cells	Median (IQR)	45 (17 – 99.5)	160.5 (91.5 – 396)	332.5 (131 –2546.5)	0.000
	Range	1 – 1019	7 – 6020	15 – 652068	
KRECs/million of cells	Median (IQR)	506.5 (36.5 – 1322)	3998 (607.5 – 7657.5)	4823 (657.5 – 18966.5)	0.000
	Range	5 – 18167	10 – 57659	23 – 9878345	

P value <0.05 is Significant. ≠: Friedman Test.

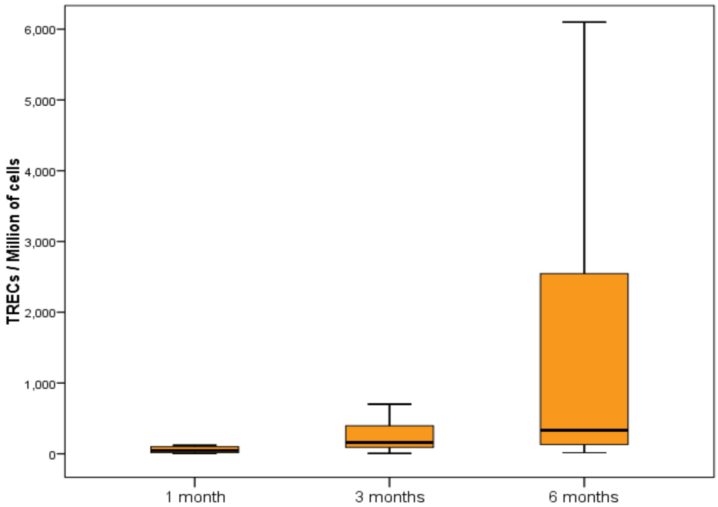

Figure 1. Boxplots representing median and interquartile ranges for TRECs/million of cells measured in HSCT patients at 1 month, 3 months and 6 months post-transplant.

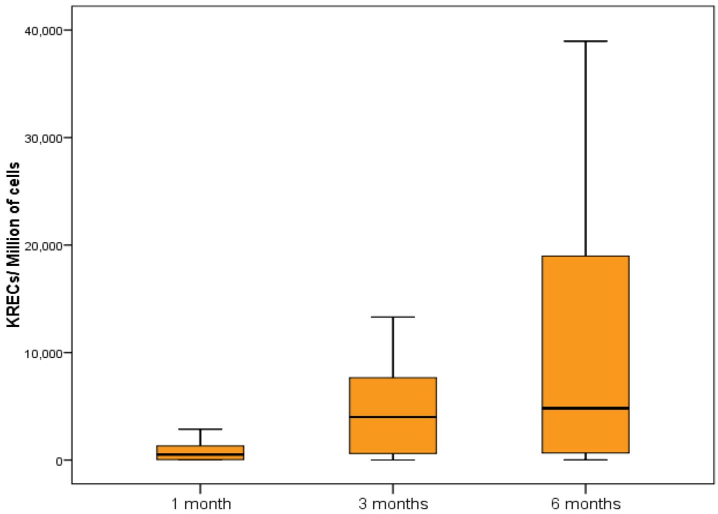

Figure 2. Boxplots representing median and interquartile ranges for KRECs/million of cells measured in HSCT patients at 1 month, 3 months and 6 months post-transplant.

Table 4. Different outcomes of transplantation of the 32 studied patients.

Observation	Item	No (%)
Relapse	No	28 (87.5%)
	Yes	4 (12.5%)
GvHD	No	19 (59.4%)
	Yes	13 (40.6%)
Survival rate	Survived	23 (71.9%)
	Died	9 (28.1%)

Table 5. Correlation of age with TRECs/million of cells and KRECs/million of cells at different times of measurement post-transplant (1, 3 and 6 months).

	Age (years)	
	r	**P* value
TRECs/million of cells (1 month)	-0.537	0.002
KRECs/million of cells (1 month)	-0.505	0.003
TRECs/million of cells (3 months)	-0.420	0.017
KRECs/million of cells (3 months)	-0.569	0.001
TRECs/million of cells (6 months)	-0.695	0.000
KRECs/million of cells (6 months)	-0.687	0.000

Spearman correlation coefficient. **P* value <0.05 is Significant.

There was no difference between females and males regarding TRECs and KRECs/million of cells as measured at 1 month, 3 months and 6 months post-HSCT (Table 6). On comparing benign and malignant groups there was a higher rate of GvHD in patients who had undergone HSCT due to malignant diseases than due to benign diseases (*P*= 0.001). However, there was no difference between the two groups in terms of relapse and survival rates (Table 7).

Table 6. Relation of gender with TRECs/million of cells and KRECs/million of cells of the studied patients at different times of measurement post- transplant (1, 3 and 6 months).

		Females	Males	**P* value
		No.= 13	No.= 19	
TRECs/million of cells (1m)	Median(IQR)	35 (3 – 87)	49 (26 – 103)	NS
	Range	1 – 116	2 – 1019	
KRECs/ million of cells (1m)	Median(IQR)	363 (43 – 2874)	742 (30 – 1316)	NS
	Range	5 – 15091	5 – 18167	
TRECs/ million of cells (3m)	Median(IQR)	159 (120 – 440)	162 (79 – 338)	NS
	Range	27 – 6313	7 – 5479	
KRECs/ million of cells (3m)	Median(IQR)	2475 (549 – 7000)	5285 (1067 – 8494)	NS
	Range	10 – 19608	24 – 57659	
TRECs/ million of cells (6m)	Median(IQR)	723 (310 – 3652)	242 (63 – 1441)	NS
	Range	90 – 24111	15 – 652068	
KRECs/ million of cells (6m)	Median(IQR)	8772 (2484 – 19523)	1803 (119 – 16803)	NS
	Range	540 – 30178	23 – 9878345	

m: month, *: Chi-square test; ≠: Mann-Whitney test *P* > 0.05 is not significant (NS)

Table 7. Comparing benign and malignant groups regarding outcomes of transplantation.

		Benign	Malignant	*P* value
		No. = 11	No. = 21	
Relapse	No	11 (100.0%)	17 (81.0%)	NS
	Yes	0 (0.0%)	4 (19.0%)	
GvHD	No	11 (100.0%)	8 (38.1%)	0.001
	Yes	0 (0.0%)	13 (61.9%)	
Survival rates	Alive	8 (72.7%)	15 (71.4%)	NS
	Died	3 (27.3%)	6 (28.6%)	

P > 0.05 is not significant (NS). ; *: Chi-square test.

Comparing benign and malignant groups regarding TRECs and KRECs measured at 1-, 3- and 6-months post-transplant revealed a statistically significant difference. Median levels of TRECs and KRECs in patients undergoing HSCT due to malignant diseases were lower as compared to those undergoing HSCT due to benign diseases (Table 8). Our study revealed that there was a statistically significant difference between patients who developed GvHD and patients who did not develop GvHD regarding TRECs, measured at 1 month and 6 months. Lower TRECs levels at 1- and 6-months post-transplant were observed in patients who developed GvHD. In addition, patients who experienced GvHD had lower levels of KRECs at 6 months than those with no GvHD (Table 9).

Table 8. Comparing TRECs/million of cells and KRECs/million of cells between both benign and malignant groups.

		Benign	Malignant	*P* value
		No. = 11	No. = 21	
TRECs/million of cells (1m)	Median (IQR)	103 (35 – 121)	31 (5 – 60)	0.003
	Range	17 – 1019	1 – 116	
KRECs/million of cells (1m)	Median (IQR)	2874 (499 – 7700)	333 (26 – 819)	0.005
	Range	20 – 18167	5 – 8680	
TRECs/ million of cells (3m)	Median (IQR)	701 (120 – 5479)	137 (89 – 279)	0.016
	Range	27 – 6313	7 – 585	
KRECs/ million of cells (3m)	Median (IQR)	8494 (4727 – 13298)	1421 (517 – 5285)	0.005
	Range	600 – 24115	10 – 57659	
TRECs/ million of cells (6m)	Median (IQR)	6100 (355 – 9959)	242 (90 – 719)	0.005
	Range	66 – 652068	15 – 6280	
KRECs/ million of cells (6m)	Median (IQR)	18410 (8772 – 36228)	1803 (540 – 6313)	0.005
	Range	405 – 9878345	23 – 71831	

P value < 0.05: Significant; ≠: Mann-Whitney test.

Table 9. Comparing TRECs/million of cells and KRECs/million levels between patients with no GvHD and patients with GvHD.

		No GvHD	GvHD	*P* value
		No. = 19	No. = 13	
TRECs/ million of cells (1m)	Median (IQR)	80 (26 – 108)	29 (5 – 60)	0.044
	Range	1 – 1019	2 – 116	
KRECs/ million of cells (1m)	Median (IQR)	742 (180 – 6020)	333 (26 – 869)	NS
	Range	7 – 18167	5 – 8680	
TRECs/ million of cells (3m)	Median (IQR)	165 (115 – 1116)	137 (43 – 279)	NS
	Range	7 – 6313	12 – 585	
KRECs/ million of cells (3m)	Median (IQR)	4866 (615 – 9318)	2113 (549 – 5729)	NS
	Range	10 – 24115	19 – 57659	
TRECs/ million of cells (6m)	Median (IQR)	1006 (240 – 8591)	179 (63 – 640)	0.027
	Range	15 – 652068	19 – 6280	
KRECs/ million of cells (6m)	Median (IQR)	8772 (2416 – 25981)	1792 (119 – 6265)	0.040
	Range	23 – 9878345	24 – 71831	

P > 0.05 is not significant (NS). ; ≠: Mann-Whitney test.

We compared TRECs and KRECs levels between patients who showed a relapse (N=4) and other patients who did not show a relapse (N=28). There was a statistically significant difference between patients who suffered from relapse and patients who did not suffer from a relapse regarding TRECs/ million of cells, measured at 6 months post-transplant only. Patients who showed a relapse had lower median levels of TRECs at 6 months (Table 10). Moreover, a statistically significant correlation was found between TRECs and KRECs, measured per million of cells at 1-, 3- and 6-months post-transplant. (Table 11)

Table 10. Comparing TRECs and KRECs levels between patients with no relapse and patients who suffered from relapse.

		No relapse	Relapse	*P* value
		No. = 28	No. = 4	
TRECs/ million of cells (1m)	Median (IQR)	45 (17 – 102.5)	47 (31.5 – 67.5)	NS
	Range	1 – 1019	29 – 75	
KRECs/ million of cells (1m)	Median (IQR)	506.5 (109 – 2101)	455.5 (28 – 992)	NS
	Range	5 – 18167	26 – 1103	
TRECs/ million of cells (3m)	Median (IQR)	163.5 (98 – 512.5)	115.5 (68.5 – 208)	NS
	Range	7 – 6313	43 – 279	
KRECs/ million of cells (3m)	Median (IQR)	2872 (607.5 – 7747)	6049.5 (2660.5 – 7564.5)	NS
	Range	10 – 57659	36 – 8315	
TRECs/ million of cells (6m)	Median (IQR)	586 (190 – 4876)	41.5 (19.5 – 186.5)	0.023
	Range	15 – 652068	19 – 310	
KRECs/ million of cells (6m)	Median (IQR)	6289 (1463.5 – 21770.5)	85.5 (38 – 5810.5)	NS
	Range	23 – 9878345	24 – 11502	

P > 0.05 is not significant (NS). ; ≠: Mann-Whitney test.

Table 11. Correlation between TRECs/million of cells and KRECs/million of cells in patients at different times of measurement post-transplant (1, 3 and 6 months).

	TRECs/million of cells					
	1 month		3 month		6 month	
	r	*P* value	r	*P* value	r	*P* value
KRECs/million of cells	0.509	0.003	0.381	0.032	0.565	0.001

P > 0.05 is not significant (NS). Spearman rank correlation coefficient.

Discussion

Dual quantitation of TRECs and KRECs for follow up and evaluation of immune reconstitution has been tested in various occasions. However, the role of TRECs and KRECs testing as predictors of outcome in HSCT still has to be studied and clarified. The aim of our work was to follow up the rate of T and B cells immune reconstitution using TRECs and KRECs after HSCT in scheduled samples at 1-, 3- and 6-months post-transplant, to correlate it with different patient parameters, and to test whether TRECs and KRECs levels would predict outcomes of transplantation. The study was conducted on 32 subjects who had undergone allogenic HSCT due to malignant and non-malignant diseases. We quantified TRECs and KRECs using a real-time polymerase chain reaction (qPCR).

In our study, we observed a decline in TRECs copies/ million of cells with increasing age. Such finding was in accordance with data reported by several studies including Douek et al., 1998,[11], Zhang et al., 1999, [13] and Mikhael & Elsorady, 2019.[14] The study by Douek et al., 1998,[11] demonstrated a sharp decline in TREC levels with age, while Zhang et al., 1999,[13] demonstrated a gradual decline in TREC levels, with stable thymic output within the first 5 years of life. We also observed a decline in KRECs copies/million of cells with increasing age which agreed with that reported by Kwok et al., 2020.[8]

Additionally, Sottini et al., 2014,[12] proposed that the number of TRECs decreased with age due to thymic involution and the number of KRECs decreased with the age with a pattern similar to TRECs only in children, whereas in the adults the bone marrow output was fairly stable throughout life. In a study by Gaballa et al., 2018,[5] the recipient age was the only significant factor associated with TRECs and KRECs levels.

Our findings showed no significant differences in TRECs and KRECs levels between females and males, regardless of age, which agreed with some previous studies that also found no differences between genders as reported by Geenen et al., 2003,[15] and by Arismendi et al., 2012.[16]

On the contrary, a study by Kwok et al., 2020, [8] observed a significantly higher level of KREC in females than males after 9–12 years indicating a slower decline in KREC levels. This may be explained by the substantial immunomodulatory role of sex hormones on immune responses [17]. However, differences in the genetic backgrounds of ethnic groups may also contribute to the contradictory findings.
Patients transplanted for malignant diseases showed significantly lower TRECs levels than benign diseases this agreed with Toubert et al., 2012,[18] and Mikhael & Elsorady, 2019,[14] who reported that thymopoiesis was significantly reduced in both newly diagnosed and chemotherapy-treated individuals with acute lymphocytic leukaemia.

Li et al., 2009,[19] and da Rocha et al., 2018,[20] have also found that patients with hematological diseases have lower TRECs numbers and poorer thymic function following treatment. Moreover, lower KRECs levels were observed in patients undergoing HSCT due to malignant diseases than benign diseases.

In terms of relapse as an outcome, TRECs and KRECs levels were compared between patients who relapsed (N=4) and those who did not (N=28). Only TRECs/million of cells measured at 6 months post-transplant revealed a statistically significant difference. Patients that relapsed reported lower levels of TRECs at 6 months post-transplant. Uzunel et al., 2014,[21] stated that low TRECs levels 2 months after transplantation were related with a high recurrence rate at 5 years, but high TRECs levels were associated with improved relapse-free survival in patients with chronic leukaemia and myelodysplastic syndrome (MDS). Mikhael and Elsorady, 2019, [14] stated that TRECs can be used to predict relapse following HSCT but not GvHD and infections. TRECs assessment was also supported by Sairafi et al., 2011,[22] as part of the normal repertoire of immunological surveillance after autologous stem-cell transplantation (ASCT). This, combined with our findings, suggests that TREC levels can be used to predict relapse.

In our study a statistically significant correlation was found between TRECs and KRECs measured at 1-, 3- and 6-months post-transplant. In a study by Gaballa et al., 2018, [5] TRECs and KRECs recovery were not correlated. Recipients either demonstrated high TRECs and low KRECs or low TRECs and high KRECs. A study by Sottini et al., 2014, [12] demonstrated that TREC and KREC recovery after HSCT in patients with primary immunodeficiency might be closely related or independent of one another. Similarly, Nakatani et al., 2014 [23] observed a group of HSCT patients who recovered KRECs but not TRECs. This heterogeneity in recovery pattern shows that transplantation-related factors may have differential effects on the thymus and BM.

Our study revealed that there was a statistically significant difference between patients who developed GvHD and patients who did not develop GvHD regarding TRECs measured at 1 month and 6 months. Lower TRECs levels at 1- and 6-months post-transplant were observed in patients who developed GvHD. In addition, patients who experienced GvHD had lower levels of KRECs at 6 months than those with no GvHD. TRECs levels may predict occurrence of GVHD and associated complications according to a report by Zhao et al., 2021,[24] which was also consistent with the findings of a study by Weinberg et al., 2001.[25]
On the contrary, Mikhael and Elsorady, 2019,[14] did not find any correlation between TRECs measured at 1 month post transplant and GvHD and observed that patients who developed GvHD had higher TRECs at this time point. In addition, Gaballa et al., 2018[5] showed that there was a significant (three-fold) increase in TREC levels in recipients with history of no GvHD compared to recipients with history of GvHD and no difference in KREC levels between the recipients with history of no GvHD compared to recipients with history of GvHD.

Finally, the present study has certain limitations. The sample size was relatively small which may have an impact on the generalization of the findings. In addition, heterogeneity of the patients was a limitation, though this is the case in many studies involving HSCT.

In conclusion, our data indicated that TRECs and KRECs levels declined with age and were affected by nature of the disease requiring HSCT being benign or malignant. TRECs and KRECs measurements can also be used to predict relapse and GvHD when measured 6-month post-transplant. In addition, our study showed that simultaneous TRECs and KRECs quantification by real time PCR can be readily adapted for clinical use, complementing analyses performed during follow-up after HSCT. Their measurement can be used as tools for immunologic monitoring after HSCT in combination with other immunologic and clinical parameters. It is a practicable, highly informative, fast, and cost-effective method.

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Author Contributions

HT and ME, Conceptualization, methodology and study design. MEL, Resources and data collection. LS, Data analysis and interpretation, writing- original draft, reviewing and editing. HG and DS, Investigation, project visualization, reviewing and editing. The authors have read and approved the manuscript.

Declaration of Conflicting Interests

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Ethical approval

The study protocol was reviewed and approved by the Research Ethics Committee, Faculty of Medicine, Ain Shams University (FMASU MD 174/ 2018).

Informed consent

A written informed consent was taken from all the patients before participation in this study.

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