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Study of different genotypes of Vitamin-D Receptor (VDR) Gene Polymorphisms in Patients Eligible for Allogeneic Hematopoietic Stem Cell Transplantation (Allo-HSCT) from a Sibling Donor

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Abstract

Background: Vitamin-D receptor (VDR) is found in both normal and neoplastic hemopoietic cells, and VDR gene polymorphism may play an important role in the clinical outcome of HLA-matched sibling donor allogeneic hematopoietic stem cell transplantation (allo-HSCT). The distribution of the genotypes in a given population would affect the impact of the polymorphism in different conditions including the clinical outcome of HSCT.

Objectives: to study the different genotypes of VDR polymorphisms in patients eligible for sibling donor allo-HSCT.

Patients and methods: Eighty-one patients undergoing allo-HSCT and their HLA-identical sibling donors were examined using polymerase chain reaction for restriction fragment length polymorphism (PCR-RFLP) analysis for FokI (rs10735810), ApaI (rs7975232) and TaqI (rs731236) polymorphisms.

Results: Patients' genotype frequencies of the three VDR polymorphisms were as follows: for FokI: Heterozygous (H) 76/78 (97.4%), mutant (M) 1/78 (1.3%) and wild (W) 1/78 (1.3%); for ApaI: (H) 26/72 (36.1%), (M) 7/72 (9.7%), and (W) 39/72 (54.2%); for TaqI: (H) 37/68 (54.4%) and (W) 31/68 (45.6%). While donors' genotype frequencies were as follows: for FokI: (W) 40/75 (53.4%), (H) 28/75 (37.3%) and (M) 7/75 (9.3%); for ApaI: (W) 31/66 (47.0%), (H) 28/66 (42.4%) and (M) 7/66 (10.6%); for TaqI: (W) 26/65 (40.0%), (H) 38/65 (58.5%) and (M) 1/65 (1.5%).

Conclusion: The genotype frequencies of VDR polymorphisms in our population were different from that of Caucasians and Asian countries, and this variability of distribution could explain that there is ethnic variability in vitamin D receptor gene polymorphisms.

Keywords: Vitamin-D Receptor (VDR), FokI (rs10735810), ApaI (rs7975232), TaqI (rs731236), Hematopoietic stem cell transplantation (HSCT).

Background:

Vitamin-D is a steroid hormone involved in bone metabolism and calcium homeostasis that has been shown to have a significant role in the regulation of host immune responses and the prevention of autoimmunity, and its action is mediated by the nuclear vitamin-D receptor (VDR) [2].

VDR is a member of the steroid/thyroid hormone receptor family. The VDR geneis located on the long arm of chromosome 12 (12q12-14) and is composed of 11 exons, the first of which is not transcribed (Figure 1) and common polymorphisms have been identified namely BsmI (rs1544410), FokI (rs10735810), ApaI (rs7975232) and Taq1(rs731236) [13].

Polymorphisms, defined as mutations with an allele frequency of at least 1% in a given population, are subtle DNA sequence variations which occur often in the population and can have modest but real biological effects. Because of their abundance in the human genome as well as their high frequencies in the human population, they have often been studied with the aim of explaining variations in the risk for common diseases. According to Li et al.(2001), humans carry a huge number of polymorphisms which may lead to different cellular effects due to various mechanisms such as enhanced/reduced transcription, altered posttranscriptional or posttranslational activity or changes in the tertiary structure of the gene product [8].

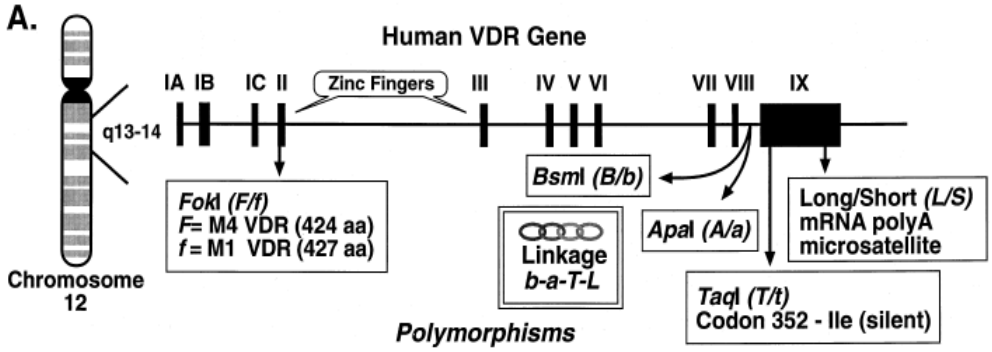

Figure (1): The human VDR chromosomal gene, containing a total of 11 exons and common polymorphisms (*Haussler et al, 1998*).

VDR is present on a wide variety of tissues outside the intestine, bones, and kidneys, which are the organs most involved in the classical role of vitamin-D. In the hematopoietic system the VDR receptor is expressed on various hematopoietic precursors as well as monocytes, some thymocytes, and activated B and T lymphocytes [6]. The VDR is found in both normal and neoplastic hemopoietic cells. It is constitutively expressed in monocytes, and in both B and T lymphocytes following activation. The variability in the distribution of the VDR genotypes among different populations may be reflected on the impact of these polymorphisms on the risk susceptibility to different autoimmune diseases and its impact on the clinical outcome of HSCT [5,9].

Aim of the work:

To study the different genotypes of VDR polymorphisms in patients eligible for sibling donor allo-HSCT.

Material and Methods:

Subjects

Between January 2014 and March 2015, a total of 81 patients; who received allogeneic stem cell transplant from fully HLA-matched siblings at the Bone Marrow Transplantation Centre, Nasser Institute Hospital, Cairo, Egypt; were recruited to participate in this study.

Inclusion criteria

Patients of both genders, aged ≥16 years, with Eastern Cooperative Oncology Group (ECOG) performance status of ≤ 2, fully HLA-identical sibling, and adequate liver and kidney functions, no medical contraindications for HSCT and cardiac ejection fraction > 60%were included in this study.

Exclusion Criteria

Patients which have not fully HLA-matched donor or have identical twin donor, pregnancy or lactation, uncontrolled infection seropositive human immunodeficiency virus (HIV) were excluded.

Genotyping of VDR gene Polymorphisms

DNA was prepared from peripheral blood samples by salting out technique (Miller et al,1988). VDR ApaI, TaqI and FokI genotypes were detected using polymerase chain reaction for restriction fragment length polymorphism (PCR-RFLP). Primer sequences are presented in Table (1), and restriction site for enzymes are presented in Table (2).

Table (1): Primer sequences for Vitamin-D receptor (VDR) gene restriction sites.

VDR site	Primer sequence
***Fok*I F**	5′-AgCTggCCCTggCACTgACTCTgCTCT-3′
***Fok*I R**	5′-ATggAAACACCTTgCTTCTTCTCCCT-C-3′
***Apa*I &*Taq* I F**	5'-CAACCAAgACTACAAgTACCgCgTCAgTgA-3'
***Apa*I &*Taq* I R**	5'-CACTTCgAgCACAAggggCgTTAgC-3'

Table (2): Sequences of Enzymes for Vitamin-D receptor (VDR) gene restriction sites.

Restriction site for enzymes	sequence
FokI	5' G G A T G N_9 ↓ 3'
	3' C C T A C N_{13} ↑ 5'
ApaI	5' G G G C C ↓ C 3'
	3' C ↑ C C G G G 5'
TaqI	5' T ↓ C G A 3'
	3' A G C ↑ T 5'

FokI (rs10735810) genotyping was performed according to Arjumand et al (2012). Digestion of the amplified 265-bp PCR product yielded two fragments with the following lengths; 169 and 96 bp. Depending on the digestion pattern, individuals were scored as FF when homozygous for the presence of the FokI site (169 and 96 bp), ff when homozygous for absence of the FokI site (265-bp) or Ff in the case of heterozygosity (265, 169 and 96 bp) [1].

ApaI ((rs7975232) and TaqI (rs731236) genotyping was determined by PCR-RFLP adapted from Sainz et al (1997). The PCR product for the ApaI and TaqI polymorphisms was 2000 bp long; the lengths of the fragments after digestion with ApaI were 1700 and 300 bp, and were 1800 and 200 bp for TaqI. Depending on the digestion pattern, individuals were scored as AA when homozygous for the absence of the ApaI site (2000-bp), aa when homozygous for presence of the ApaI site (1700-bp& 300-bp) or Aa in the case of heterozygosity (2000, 1700 and 300-bp), and scored as TT when homozygous for the absence of the TaqI site (2000 bp), tt when homozygous for presence of the TaqI site (1800-bp &200 bp) or Tt in the case of heterozygosity (2000, 1800 and 200-bp).

After digestion for 1 hour at 370C using Apa1 and taq1 restriction enzymes respectively, the genotypes were defined as A,T (indicating the absence of the restriction site) or a, t (indicating the presence of the restriction site). The genotype frequency of the three VDR gene polymorphisms; FokI, ApaI and TaqI; are demonstrated as Wild "W"; (W = FF, AA, TT, respectively), Heterozygous "H"; (H = Ff, Aa, Tt, respectively) and Homozygous Mutant "M"; (M = ff, aa, tt, respectively).

Statistical analysis

Data was entered on a PC using Excel 2013 (Microsoft Corporation, USA) and was analyzed using SPSS version 21 (IBM Inc., USA). Data were described as numbers and frequencies (percentages).

Results:

A total of 81 patients (59 males and 22 Females) with various hematologic disorders underwent allogeneic HSCT. Patients' characteristics are shown in Table (3). The age of the patients at time of transplantation ranged from 16-56 with a mean of 31.79 ± 9.28 and a median of 31years. Twenty-two patients (27.2%) were between 16 and 25, while 59 patients (72.8%) were > 25 years.

Table (3): Characteristics of 81 patients subjected to allogeneic HSCT from an identical sibling

Parameter		N	%
Age: yrs	16 - 25	22	27.2 %
	> 25	59	72.8 %
Gender	Male	59	72.8 %
	Female	22	27.2 %
Diagnosis	AML	36	44.4 %
	SAA	22	27.2 %
	ALL	11	13.6 %
	CML	5	6.2 %
	MDS	3	3.7 %
	Biphenotypic	1	1.29 %
	CLL	1	1.29 %
	Myelofibrosis	1	1.29 %
	PNH	1	1.29 %
Conditioning Regimen	Bu/Cy	41	50.6 %
	Flu/Cy	23	28.4 %
	TBI/Cy	10	12.3 %
	Others	7	8.6 %
GVHD Prophylaxis	CSA + MTX	78	96.3 %
	CSA + MMF	3	3.7 %
CMV Status	- ve (≤ 15 AU/ml)	2	2.5 %
	+ ve (<250)	57	70.4 %
	+ ve (≥250)	22	27.2 %
HCV status	- ve	70	86.4 %
	+ ve	11	13.6 %

GVHD = Graft versus host disease, **CMV** = Cytomegalovirus, **HCV** = Hepatitis-C virus, **Bu/Cy** = busulphan and cyclophosphamide, **Flu/Cy** = fludarabine and cyclophosphamide, **TBI/Cy** = total body irradiation and cyclophosphamide, **AML** = acute myeloid leukemia, **ALL** = acute lymphoblastic leukemia, **SAA** = severe aplastic anemia, **CML** = chronic myeloid leukemia, **MDS** = myelodysplastic syndrome, **CLL** = chronic lymphocytic leukemia, **PNH** = paroxysmal nocturnal hemoglobinuria).

The diagnoses were acute myeloid leukemia (AML) (44.4%), severe aplastic anemia (SAA) (27.2%), acute lymphoblastic leukemia (ALL) (13.6%), chronic myeloid leukemia (CML) (6.2%), myelodysplastic syndrome (MDS) (3.7%), biphenotypic acute leukemia (BAL) (1.2%) and others (3.7%) including one case of chronic lymphocytic leukemia (CLL), one case of primary myelofibrosis (PMF) and one case of paroxysmal nocturnal hemoglobinuria (PNH).

All patients received stem cells from HLA-identical siblings and in all patients, the stem cell source was granulocyte-colony stimulated factor (G-CSF)-mobilized peripheral blood stem cells.

Forty-one patients (50.6 %) received conditioning with busulphan and cyclophosphamide (Bu/Cy), 23 (28.4%) received fludarabine and cyclophosphamide

(Flu/Cy), 10 (12.3%) received total body irradiation and cyclophosphamide (TBI/Cy), and 7 patients (8.6%) received other regimens such as fludarabine and melphalan (Flu/Alk), fludarabine and busulphan (Flu/Bu) or fludarabine, busulphan and antithymocyte globulin (Flu/Bu/ATG).

The mean age of the donors was 29.17 ± 9.96 years. Donor–recipient gender matching is shown in Table (3).

Patient and Donor genotype frequencies of the three VDR polymorphisms are presented in Table (5); for FokI: Heterozygous (H) 76/78 (97.4%), mutant (M) 1/78 (1.3%) and wild (W) 1/78 (1.3%); for ApaI: (H) 26/72 (36.1%), (M) 7/72 (9.7%), and (W) 39/72 (54.2%); for TaqI: (H) 37/68 (54.4%) and (W) 31/68 (45.6%). While donors' genotype frequencies were as follows: for FokI: (W) 40/75 (53.4%), (H) 28/75 (37.3%) and (M) 7/75 (9.3%); for ApaI: (W) 31/66 (47.0%), (H) 28/66 (42.4%) and (M) 7/66 (10.6%); for TaqI: (W) 26/65 (40.0%), (H) 38/65 (58.5%) and (M) 1/65 (1.5%).

Table (4): Characteristics of 81 donors of allogeneic HSCT to an identical sibling

Parameter		N	%
Gender	Male (M)	52	64.2 %
	Female (F)	29	35.8 %
Donor/Patient	M - M	40	49.4 %
Gender	M - F	12	14.8 %
Mismatch	F - M	7	8.6 %
	F – F	22	27.2 %

M - M = male to male, **M - F** = male to female, **F - M** = female to male, **F - F** = female to female.

Table (5): Genotype frequencies in Patients and Donors subjected to allogeneic HSCT from an identical sibling

Genotype	Patient			Donor		
	FokI	***ApaI***	***TaqI***	***FokI***	***ApaI***	***TaqI***
H	76 (97.4%)	26 (36.1%)	37 (54.4%)	28 (37.3%)	28 (42.4%)	38 (58.5%)
M	1 (1.3%)	7 (9.7%)	0 (0.0%)	7 (9.3%)	7 (10.6%)	1 (1.5%)
W	1 (1.3%)	39 (54.2%)	31 (45.6%)	40 (53.4%)	31 (47.0%)	26 (40.0%)
Total	78 (100%)	72 (100%)	8 (100%)ך	75 (100%)	66(100%)	65 (100%)

(**H**: Heterozygous, **M**: Mutant/Homozygous, **W**: Wild).

Discussion:

In our study the genotype frequency of the three VDR gene polymorphisms; FokI, ApaI and TaqI; was demonstrated as (W = ff, AA, TT, respectively), (H = Ff, Aa, Tt, respectively) and (M = FF, aa, tt, respectively). Recipient genotype frequencies of the three VDR polymorphisms were; (FF = 1.3%, Ff = 97.4%, ff =1.3%, AA = 54.2%, Aa= 36.1%, aa = 9.7%, and TT = 45.6%, Tt = 54.4%, tt = 0.0%).

In our study the frequencies of the VDR polymorphisms vary between our population and others; where Middletonet al (2002), in UK revealed that the genotype frequencies were (F = 64%, f =37%, A = 48%, a = 52%, and T = 64%, t = 36%) [9]. Bogunia et al (2008), in Poland revealed that the genotype frequencies were (FF = 26%, Ff = 55%, ff =16%, AA = 26%, Aa= 48%, aa = 26%, and TT = 33%, Tt = 55%, tt = 12%) [4]. Cho et al (2012), in Korea revealed also different genotype frequencies which were (AA = 8.8 %, Aa= 11.6 %, aa = 50.3 %, and TT = 84.4 %, tt = 12.9 %) [5]. Minamitani et al (1998) in Japan showed that the genotype frequencies were (FF = 37%, Ff = 51%, ff = 12% and TT = 77%, Tt = 22%, tt = 1% respectively) [11]. Bid et al (2005) in North Indian population revealed that the genotype frequencies were (FF= 44%, Ff = 49%, ff = 7% and TT = 49%, Tt = 40%, tt = 11% respectively) [3]. Also Kung et al (1998) in Chinese population recorded that the "A" allele of the ApaI polymorphism is only found in 5% of that population.

We can conclude that the frequency of the polymorphisms is dependent on ethnicity. It is likely that this difference could alter the influence of polymorphism on the susceptibility to diseases diluting the effects observed in other populations. This also applies to the impact of these polymorphisms on the outcome of HSCT. Hence it is essential to perform such studies in each community; results from one population cannot be extrapolated to others.

Conflict of Interests:

The authors declare that they have no conflict of interests.

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