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Research Article

Frequency and Distribution of Fok1rs 2228570 of Vitamin D Receptor Gene among Healthy Sudanese Population

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Abstract

Background: Vitamin D plays a role in critical cellular processes such as apoptosis, cell development, and division. The Fok1 polymorphism in the VRD gene is T/C resulting in larger vitamin D receptor protein with lesser expression activity. Fok1 polymorphism is thought to be linked to many diseases including diabetes mellitus, cancers, and rheumatoid arthritis. The snipe also provides effective and sensitive diagnostic and predictive tools and affected by differences in ethnicity and individuals' life style. This study reports on the frequency and distribution of Fok1 SNP among healthy Sudanese transplantation donors.

Method: This cross sectional study was conduct in National Cancer Institute University of Gezira, Sudan. One hundred and forty six healthy Sudanese transplantation donors were enrolled in this study. Two ml of venous blood was collected from each participant and stored at -20 till DNA extraction. Qaigen kit was used for DNA extraction. VDR Fok1 was genotyped by CTPP-PCR with proper primers designing and PCR condition. Data was collected by structured questionnaire and analyzed by SNP Stats online tool and medical calculator.

Results: According to study results theFok1 polymorphism distributed among Sudanese with percentage of 49.9% (TT + CT), frequency of SNP variants was 69.8% regarding C allele and 30.2% for T allele, while the three genotypes was 10.2% for TT, 39.7% for TC and 50.1% for CC genotype.

Conclusion: The frequency of Fok1 VDR SNP was high among healthy Sudanese transplantation donors, this necessitate the conduction of wide area survey among other healthy Sudanese population. This intern will help in investigating the nature of the relation of this SNP with other different diseases.

Keywords: Vitamin D, Fok1rs. 2225870, VDR SNP, Sudanese

INTRODUCTION

The action of bioactive form of vitamin D, 1,25-dihydroxyvitamin D3, is regulated by the vitamin D receptor (VDR).This nuclear receptor is a ligand-dependent transcription factor. (Rashid, Muzaffar et al. 2015). The VDR gene is found on the chromosome12q13.11 (Al Safar, Chehadeh et al. 2018). The VDR is expressed in almost all tissues and cells in the body, including the brain, skin, breast, prostate, colon, and activated T and B lymphocytes. 1,25(OH)2D is currently regarded as one of the most powerful hormones for controlling cell development and maturation. More than 2000 genes are thought to be affected by the vitamin action, either directly or indirectly.(Gnagnarella, Raimondi et al. 2020),it is plays a role in calcium homeostasis as well as critical cellular processes such as apoptosis, cell development, and division (Shahabi, Alipour et al. 2018). Within the DNA-binding site of the VDR gene, the Fok1 polymorphism is located near the 5-UTR sequence (Neela, Suryadevara et al. 2015), it is happened due to thymine/cytosine change, which converts an ACG codon ten base pairs upstream from the translation start codon to an ATG codon, leading to the creation of a second start codon. Three extra amino acids will be added to the VDR protein, resulting in a bigger VDR protein. The Fok1 polymorphism's “f “ variant has reduced transcriptional activity than the “F” variant. (Khan, Bielecka et al. 2014). Additionally, changes in VDR expression and activity might result in decreased vitamin D utilization, metabolism, and blood levels of bio-active vitamin D. (El-Shorbagy, Mahmoud et al. 2017)

According to (Hitchon, Sun et al. 2012), the binding sites for vitamin D receptors are abundant in gene loci linked to autoimmunity and rheumatoid arthritis (RA). Moreover, VitD insufficiency and altered VitD receptor function both have been related to increased vulnerability to infection and cancer. In certain groups, only the Fok1 VitD receptor polymorphism has been linked to the development of RA.(Hitchon, Sun et al. 2012). FokI was reported among a lot of studies that detected VDR gene polymorphisms in cancer

patients namely Bsml, ApaI and TaqI. (Beysel, Eyerci et al. 2018).VDR BsmI, ApaI, and FokI gene polymorphisms have shown to increase the risk of developing breast cancer (Al-Janabi, Algenabi et al. , Iqbal and Khan 2017, Raza, Dhasmana et al. 2019). Furthermore, FokI polymorphism has been also linked to an increased risk of ovarian cancer (Liu, Li et al. 2013). T/T genotype of Fok1 was linked to an increased progress in squamous cell carcinoma of the head and neck (Hama, Norizoe et al. 2011).Many studies have also shown a link between vitamin D deficiency and impaired glucose tolerance, as seen in type 2 diabetes, this is due to either activation of the Vitamin D receptor gene directly or indirect interaction of vitamin D with calcemical hormones or inflammation (Al-Shoumer and Al-Essa 2015)(Sung, Liao et al. 2012).Vitamin D3 possesses immune modulatory effects and regulates insulin production.(Kaftan, Hussain et al. 2021). Few studies in Sudan concerning the VDR gene polymorphisms and their role in healthy and diseased population, the documented reports came from studies cited by (Yousif, Ismail et al. 2017)(Alkareem and Hussien 2017)(Ibrahim, Ali et al. 2015), the authors highlight the association of VDR gene polymorphisms with development of breast cancer, diabetes and hematological malignancies. This study attempts to determine the frequency and distribution of Fok1polymorphism among healthy Sudanese transplantation donors

METHODS

Study subjects:-

This cross sectional study conduct in National Cancer Institute (NCI), Gezira state Sudan, the study involved146 healthy transplantation donors (45 male and 101 females) at different ages ranges from 25 to 55 years old. NCI is referral natural teaching and research hospital in Sudan, is concerns with diagnosis and treatment of different types of cancers, the institute and due to it is high capability is also responsible for tissue typing specially for renal transplantation. The recruitment of participants was done after through clinical examination and tough laboratory confirmation to ensure their medicals fitness, and after giving their verbal consent.

Sample collection and DNA extraction:

2.5 ml of venous blood was collected from each participant using a sterile syringe and proper sample collection procedure, then discarded into a labeled EDITA blood container and Stored at -20 until extraction. DNA was extracted by Qiagen extraction kit according to manufacture instruction; both concentration and purity of extracted **DNA** were measured and adjusted.

Fok1VDR SNPs was genotyped by **CTPP-PCR** using primers newly designed listed in the table (1). The amplification was carried out in a 15-µL reaction mixture containing 5µL of 50 ng template DNA, 0.5µL of common primers (CP570T, CP570C) and 1 µL allele specific primer (AP570T, AP570C) (10 pmol), and 7µL master mix Taq polymerase. The PCR was performed under the following conditions: initial denaturation at 95°C for 3 min then denaturation at 94°C for 60 s, followed by annealing at 55°C45 s, 72°C for 45 s extension for 40 cycles and final elongation at 72°C for 3 min while final hold at - 4°C. The PCR products were visualized using 2% agarose gel containing (10 µL) ethidium bromide under ultraviolet trans illumination. The different sizes of DNA fragments were as follows: 240bpin case of T allele and 169bp for C allele while the Common band (internal control) was 390bp.

Table 1: Primers sequence and PCR Product size:

Primer sequence	Product size
AP570T: 5- CTGGCCGCCATTGCCTtCA – 3	240 bp (T allele)
CP570T: 5- CCAGGCAGCTGATTCCAAG -3	
AP570C:5- GCTTGCTGTTCTTACAGGtAC–3	169 bp (C allele)
CP570C: 5- TCACCTGAAGAAGCCTTTGC -3	Common 390 bp

RESULT AND DISCUSSION:

Table 2: Alleles and genotypes frequencies of Fok1polymorphism among study population

	Count	Frequency
Alleles		
C	204	69.8%
T	88	30.2%
Genotypes		
CC	73	50.1%
CT	58	39.7%
TT	15	10.2%

Table 3: comparison of genotypes and alleles frequency of VDR gene (FOK I) polymorphism of Sudanese population with various populations

		Genotypes				Alleles			
Ethnicity	**NO.**	**CC**	**CT**	**TT**	**P. value ***	**Major**	**Minor**	**P. value**	**Reference**
Sudan	146	50	40	10	Ref			Ref	Current study
Americans									
Black Pennsylvania	104	63	31	6	0.1623	78	22	NS	(Zmuda, Cauley et al. 2000)
Mexican, California	100	37	48	15	0.1596	61	39	NS	(Gross, Eccleshall et al. 1996)
Asia									
North India	346	44	49	7	0.4020	68.5	31.5	NS	(Bid, Mishra et al. 2005)
South India	80	43	29	8	0.8676	72	28	NS	(Selvaraj, Chandra et al. 2003)
Japan	249	37	51	12	0.1778	62	38	NS	(Minamitani, Takahashi et al. 1998)
Taiwan	101	36	49	15	0.1231	61	39	NS	(Cheng and Tsai 1999)
Europe									
Finland	144	28	58	14	0.0062	60	40	NS	(Videman, Leppävuori et al. 1998)
France	100	43	47	10	0.5798	66	34	NS	(Correa-Cerro, Berthon et al. 1999)
England	108	48	41	11	0.9509	69	31	NS	(Hutchinson, Osborne et al. 2000)
Middle East									
Jordan	126	32	48	20	0.0182	56.4	43.6	NS	(Karasneh, Ababneh et al. 2013)
UAE	281	38	42	20	0.0182	59.2	40.8	NS	(Osman, Al Anouti et al. 2015)
Syria	78	36	58	6	0.0372	65	35	NS	(HADDAD 2021)
South Pacific									
Australia	577	37	48	15	0.1596	61	39	NS	(Kotowicz, Pasco et al. 1998)

* = Significant p < 0.05 NS= not significant

DISCUSSION

The biological role of Vitamin D is not only limited to regulating calcium metabolism and bone health, but also extends to the regulation of many genes that directly or indirectly affect in the incidence rates of many diseases that include different types of cancer, such as breast cancer, prostate, kidney. The Fok1SNP VDR leads to creation of a second start codon resulting in a bigger VDR protein with reduced transcriptional activity.(Khan, Bielecka et al. 2014).

Many studies documented a strong relationship between genetic changes that occur in the vitamin D receptor gene and a lot of diseases, including diabetes, rheumatism, autoimmune diseases.(Hitchon, Sun et al. 2012)(Al-Shoumer and Al-Essa 2015).The aim of this study is to determine the prevalence of Fok1 SNP variants in healthy Sudanese population because of its greater importance in influencing the effectiveness of the vitamin D receptors action, as well as comparing our Sudanese with other corresponding healthy populations worldwide, to find out the ethnic differences and the extent of their impact on the spread of the mutation, which in turn may lead to various serious consequences, we have touched on some of them previously.

The recruited subjects in this study were those who come to NCI for histocompatibility testing for the purpose of donation, their selection was confirmed after through clinical examination and laboratory diagnosis (CBC, RFT, LFT, UG, viral screening against hepatitis B, C and HIV, hormonal assay for cortisol and TFT abdominal ultrasonography, CT scan, MR), to ensure their medical fitness.

In General, SNPs provide an effective and sensitive diagnostic and predictive tool, because they are easy to be track and detect. Their diagnostic efficiency can be used to trace the differences in ethnicity and individuals' life style, so reflects the changes in pathological patterns of diseases.

Despite the sharp ethnic diversity in Sudan and the environmental differences in the standard and lifestyle among individuals, there are no records and genetic databases that reflect this diversity.

The use of the CTPP–PCR technique in this study was able to detect the presence of Fok1 SNP VDR among healthy Sudanese transplantation donors. Other studies also proved detection of Fok1 SNP VDR using other SNP detection techniques viz RFLP (Bid, Mishra et al. 2005)real time PCR (Osman, Al Anouti et al. 2015). The current study revealed that half of healthy transplantation donors participants carrying the SNP in form of hetero and homo mutant genotypes (CT+TT), this findings was confirmed by other studies done elsewhere (Correa-Cerro, Berthon et al. 1999)(Minamitani, Takahashi et al. 1998). However, some studies carried in Jordan (Karasneh, Ababneh et al. 2013),Finland (Videman, Leppävuori et al. 1998), Syria (HADDAD 2021) and UAE(Osman, Al Anouti et al. 2015) gave different percentages.

According to our findings the frequency of Fok1 SNP VDR genotypes were 10.2% for TT 39.7% for TC and 50.1% for CC genotype, which compared with different populations and ethnic groups from some countries (table 3).Significant (p. value < 0.05) variation of SNP distribution was only observed within the Middle East group (Jordan, UAE and

Syria) and from European countries only Finland. This may attributed to the variation in the life style factors and population diversity in Sudan when compared to these countries.

This study is the first of its kind in Sudan, and we believe that it will be a starting point for establishing genetic databases for healthy Sudanese, expanding the study of mutations and genetic changes and to highlight their relationship to diseases susceptibility.

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REFERENCES:

1. Al-Janabi, A. M., et al. "Association of Vitamin D Receptor-FOKI Gene Polymorphism with Breast Cancer Risk in Iraqi Female Patients."
2. Al-Shoumer, K. A. and T. M. Al-Essa "Is there a relationship between vitamin D with insulin resistance and diabetes mellitus?" World journal of diabetes 2015; 6(8):1057. https://doi.org/10.4239/wjd.v6.i8.1057
3. Al Safar, H., et al. "Vitamin D receptor gene polymorphisms among Emirati patients with type 2 diabetes mellitus." The Journal of steroid biochemistry and molecular biology 2018; 175:119-124. https://doi.org/10.1016/j.jsbmb.2017.03.012
4. Alkareem, A. and S. H. Hussien, Assessment of Serum 25 (OH) Vitamin D Concentration and Bsml Vitamin D Receptor Polymorphism in Sudanese Patients with Type 2 Diabetes Mellitus, Khartoum Teaching Hospital, Sudan,(2015–2016), University of Gezira. 2017
5. Beysel, S., et al. "VDR gene FokI polymorphism as a poor prognostic factor for papillary thyroid cancer." Tumor Biology 2018; 40(11):1010428318811766. https://doi.org/10.1177/1010428318811766
6. Bid, H. K., et al. "Vitamin-D receptor (VDR) gene (Fok-I, Taq-I and Apa-I) polymorphisms in healthy individuals from north Indian population." Asian Pac J Cancer Prev 2005; 6(2):147-152.
7. Cheng, W.-C. and K. Tsai, "The Vitamin D Receptor Start Codon Polymorphism (Fok 1) and Bone Mineral Density in Premenopausal Women in Taiwan." Osteoporosis international 1999; 9(6):545-549. https://doi.org/10.1007/s004390051102
8. Correa-Cerro, L., et al. "Vitamin D receptor polymorphisms as markers in prostate cancer." Human genetics 1999; 105(3):281-287.
9. El-Shorbagy, H. M., et al. "Association of vitamin D receptor gene polymorphisms with breast cancer risk in an Egyptian population." Tumor Biology 2017; 39(10):1010428317727738. https://doi.org/10.1177/1010428317727738
10. Gnagnarella, P., et al. Vitamin D Receptor Polymorphisms and Cancer. Sunlight, Vitamin D and Skin Cancer, Springer: 2020; 53-114.
11. Gross, C., et al. "The presence of a polymorphism at the translation initiation site of the vitamin D receptor gene is associated with low bone mineral density in postmenopausal Mexican-American women." Journal of Bone and Mineral Research 1996; 11(12):1850-1855. https://doi.org/10.1002/jbmr.5650111204
12. HADDAD, S. "Vitamin-D receptor (VDR) gene polymorphism (FokI) in Syrian healthy population." Gene Reports 2021; 23:101083. https://doi.org/10.1016/j.genrep.2021.101083
13. Hama, T., et al. "Prognostic significance of vitamin D receptor polymorphisms in head and neck squamous cell carcinoma." PLoS One 2011; 6(12):e29634. https://doi.org/10.1371/journal.pone.0029634
14. Hitchon, C. A., et al. "Vitamin D receptor polymorphism rs2228570 (Fok1) is associated with rheumatoid arthritis in North American natives." The Journal of rheumatology 2012; 39(9):1792-1797. https://doi.org/10.3899/jrheum.120387
15. Hutchinson, P. E., et al. "Vitamin D receptor polymorphisms are associated with altered prognosis in patients with malignant melanoma." Clinical Cancer Research 2000; 6(2):498-504.
16. Ibrahim, Y. A., et al. "Association of Vitamin D Receptor (VDR) Start Codon FokI Polymorphism with Acute Myelogenous Leukemia." European Academic Research 2015; 2:15474-15482.
17. Iqbal, M. u. N. and T. A. Khan "Association between vitamin D receptor (Cdx2, Fok1, Bsm1, Apa1, Bgl1, Taq1, and Poly (A)) gene polymorphism and breast cancer: a systematic review and meta-analysis." Tumor Biology 2017; 39(10):1010428317731280. https://doi.org/10.1177/1010428317731280
18. Kaftan, A. N., et al. "Association of sunshine vitamin receptor gene polymorphisms (rs 2228570) and (rs7975232) with the type 2 diabetes mellitus in Iraqi patients from the middle Euphrates region." Gene Reports 2021; 22:100977. https://doi.org/10.1016/j.genrep.2020.100977
19. Karasneh, J. A., et al. "Association of vitamin D receptor gene polymorphisms with chronic and aggressive periodontitis in Jordanian patients." European journal of oral sciences 2013; 121(6):551-558. https://doi.org/10.1111/eos.12085
20. Khan, M. I., et al. "Vitamin D receptor gene polymorphisms in breast and renal cancer: current state and future approaches." International journal of oncology 2014; 44(2):349-363. https://doi.org/10.3892/ijo.2013.2204
21. Kotowicz, M., et al. "Vitamin D receptor start codon polymorphism is not associated with bone mineral density in Australian women." Bone 1998; 23:S372.
22. Liu, Y., et al. "Polymorphisms in the vitamin D receptor (VDR) and the risk of ovarian cancer: a meta-analysis." PLoS One 2013; 8(6):e66716. https://doi.org/10.1371/journal.pone.0066716
23. Minamitani, K., et al. "Difference in height associated with a translation start site polymorphism in the vitamin D receptor gene." Pediatric research 1998; 44(5):628-632. https://doi.org/10.1203/00006450-199811000-00002
24. Neela, V. S. K., et al. "Association of Taq I, Fok I and Apa I polymorphisms in Vitamin D Receptor (VDR) gene with leprosy." Human immunology 2015; 76(6):402-405. https://doi.org/10.1016/j.humimm.2015.04.002
25. Osman, E., et al. "Frequency of rs731236 (TaqI), rs2228570 (Fok1) of Vitamin-D Receptor (VDR) gene in Emirati healthy population." Meta gene 2015; 6:49-52. https://doi.org/10.1016/j.mgene.2015.09.001
26. Rashid, M. U., et al. "Association between the BsmI Polymorphism in the Vitamin D receptor gene and breast cancer risk: Results from a Pakistani case-control study." PLoS One 2015; 10(10). https://doi.org/10.1371/journal.pone.0141562
27. Raza, S., et al. "Molecular mechanism of cancer susceptibility associated with Fok1 single nucleotide polymorphism of VDR in relation to breast cancer." Asian Pacific Journal of Cancer Prevention: APJCP, 2019; 20(1):199. https://doi.org/10.31557/APJCP.2019.20.1.199
28. Selvaraj, P., et al. "Association of vitamin D receptor gene variants of BsmI, ApaI and FokI polymorphisms with susceptibility or resistance to pulmonary tuberculosis." Current Science: 2003; 1564-1568.
29. Shahabi, A., et al. "Vitamin D receptor gene polymorphism: association with susceptibility to early-onset breast cancer in Iranian, BRCA1/2-mutation carrier and non-carrier patients." Pathology & Oncology Research 2018; 24(3): 601-607. https://doi.org/10.1007/s12253-017-0281-8
30. Sung, C.-C., et al. "Role of vitamin D in insulin resistance." Journal of biomedicine and biotechnology. 2012. https://doi.org/10.1155/2012/634195
31. Videman, T., et al. "Intragenic polymorphisms of the vitamin D receptor gene associated with intervertebral disc degeneration." Spine, 1998; 23(23):2477-2485. https://doi.org/10.1097/00007632-199812010-00002
32. Yousif, S. O., et al. Vitamin D receptor (VDR) gene polymorphisms among Sudanese females with breast cancer attending Radio and Isotope Center of Khartoum, AACR. 2017. https://doi.org/10.1158/1538-7445.AM2017-1455
33. Zmuda, J. M., et al. "Molecular epidemiology of vitamin D receptor gene variants." Epidemiologic reviews, 2000; 22(2):203-217. https://doi.org/10.1093/oxfordjournals.epirev.a018033