Vitamin D status and its relation with insulin resistance and VDR-FokI polymorphism in Iranian non-melanoma skin cancer (NMSC) patients: a case-control study

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Abstract

Background Sunlight exposure, the main source of endogenous vitamin D synthesis, may increase the risk of non-melanoma skin cancer (NMSC) development. Vitamin D receptor (VDR) polymorphisms are associated with 25(OH)D levels, cancer development and insulin resistance. Objective This study aimed to examine the associations among vitamin D status, VDR FokI polymorphism, insulin resistance and NMSC. Methods This case-control study included 73 diagnosed cases of NMSC and 72 healthy controls from dermatology clinics at Razi Hospital, Tehran, Iran. A questionnaire was used to assess sunlight exposure. The extracted DNA from whole blood samples were genotyped. Fasting serum 25-hydroxyvitaminD (25(OH)D)), lipid profile, glucose, and insulin were measured. To evaluate insulin resistance, HOMA-IR formula was used. Results We found a significant higher duration of cumulative sunlight exposure in cases compared with controls ($p<0.001$). However, 25(OH)D concentrations were not significantly different between cases and controls (30±15 vs. 29±15 ng/mL, $p=0.78$). Higher levels of insulin ($p = 0.004$) and HOMA-IR score ($p= 0.019$) were observed in Ff and ff genotype of FokI. We did not observe any significant increased risk of NMSC due to f allele, as compared with FF (OR =2.33, 95% CI 0.81-6.75, $p=0.12$). The components of lipid profile, fasting serum glucose, iPTH and anthropometric measures did not differ significantly across VDR genotypes. Conclusion In conclusion, sunlight exposure was associated with NMSC risk. VDR FokI polymorphisms appears to influence insulin resistance in the NMSC patients.

Introduction

Suboptimal vitamin D status in many countries among different age and sex groups is considered a serious global health problem. The importance of vitamin D deficiency (VDD) is not confined to the deleterious effects on musculoskeletal system. A growing body of evidence shows the predisposing effect of VDD in many human pathologies including cardiovascular disease, diabetes, autoimmune disorders(1), and certain cancers (1, 2). Skin cancers are among the most common types of human neoplasms (3). In Iran, the incidence of skin cancers is on a rise (4) and they account for approximately 15% of all types of cancer (5).

Exploration of vitamin D receptor (VDR) on a vast variety of tissues and cells including basal cell and squamous cell carcinomas indicated new roles for this vitamin (6). Although a strong body of evidence indicates a protective role for vitamin D against colorectal, breast, prostate, and pancreatic cancers, the association of vitamin D and skin cancers is intriguing. While solar ultra violet beam (UVB) is the major source of vitamin D in the nature, direct exposure to the same wavelength of UVB has been known as the main culprit in development of skin cancers. Therefore, it is speculated that compared to the general population, patients with skin cancer may have longer periods of sun exposure and thus higher vitamin D status.

The possible link between insulin resistance (IR) and human cancers, including skin malignancies, can be a new argument (7). The associations between IR and several malignancies including colon, liver,

pancreas(8), endometrium(9), breast (10), lung (11) and thyroid cancers (12) have been reported. The ameliorating effect of vitamin D on IR has been shown by clinical trials (13). Whether this effect can mediate any anti-cancer property of vitamin D is still unknown.

Despite previous studies on the relationship between 25(OH)D3 and IR, there is still gap in determination of the molecular mechanisms through which VDR affects IR (14-16). There is limited evidence that VDR gene variants BsmI and FokI might affect BMI, IR, and serum HDL-cholesterol. (16-19). However, scant evidence is available regarding this association in cancer patients.

The nutrigenetic effect of VDR polymorphisms on response to vitamin D intake has been already documented (20). VDR polymorphism is characterized by altered expression levels (21, 22) leading to decrease or increase in vitamin D activity (21). FokI polymorphism is the only known polymorphism that generates an altered protein (20, 23, 24). VDR polymorphisms have been linked to risk of several types of cancer, including prostate, breast, bowel (25 , 26) and skin malignancies (27 , 28). However, the association of VDR variants and risk of skin cancer has not been understood clearly yet (29). Data from a meta-analysis suggest that there might be a possible positive link between VDR FokI and BsmI polymorphisms and cutaneous malignant melanoma (CMM) and non-melanoma skin cancer (NMSC) risks (30). However, results of a systematic review did not show any significant association between VDR variants of TaqI, BsmI and FokI and risk of NMSC (29). With regard to the possible role of vitamin D in skin cancers and notably NMSCs, several issues might be raised: (1) Do NMSC patients have longer duration of occupational direct sun exposure than unaffected people? and if yes, (2) Does it have any influence on their vitamin D status? in other words, do NMSC patients have higher vitamin D status compared to the unaffected people as reported by some studies (31, 32)? (3) Is there any association between IR and NMSC? and finally, (4) Is there any association among VDR FokI polymorphisms, vitamin D status and NMSC risk? We aimed to address the above-mentioned issues in this hospital-based case-control study.

Subjects And Methods

Participants and Clinical Samples

From September 2016 to April 2018, a total of 130 individuals were enrolled in this case-control study. Cases were 73 basal cell carcinoma (BCC) or squamous cell carcinoma (SCC) patients recruited from the dermatology clinics at Razi Hospital of Tehran University of Medical Sciences. BCC or SCC needed to be diagnosed within three months before the time of recruitment.

The controls were 72 unrelated healthy volunteers who were matched for age and sex to the patients with NMSC (Table I). The following criteria rendered individuals ineligible to enter this study: taking any nutritional supplements containing vitamin D, calcium, omega-3 fatty acids, and antioxidants for at least 3 months preceding the time of recruitment; medications that modify vitamin D metabolism, including but not limited to corticosteroids, estrogens, and calcitonin for at least 3 months preceding the time of recruitment; history of any other cancer, renal or liver diseases. Participants completed a questionnaire

including information on marital status, education level, medication and supplement use history, disease history, sunscreen use, and average hours of sun exposure per day. Fasting blood samples were obtained from all participants for DNA genotyping and biochemical analyses. The study procedures were approved by the Research Council and the Ethics Committee of the Iranian National Nutrition and Food Technology Research Institute (NNFTRI). All participants gave consent by signing a written consent form.

Anthropometry and blood pressure

Participants were weighed with minimal clothing, wearing light clothes and no shoes using a digital scale (Seca 808; Seca, Hamburg, Germany). Height was measured by a stadiometer (Seca 216, Seca, Hamburg, Germany). The degree of accuracy for weight and height was 0.1 kg and 0.1 cm respectively. BMI was calculated as the weight in kilograms divided by the square of the height in meters (kg/m^2). Based on the 2004 World Health Organization (WHO) classifications, categories of BMI were <18.5, 18.5-24.9, and >25.0 representing underweight, normal weight, and overweight respectively.

A flexible tape measure was used to determine the hip circumference (HC) and the waist circumference (WC) with degree of accuracy of 0.1 cm. For WC, the tape measure was placed on the approximate midpoint between the last palpable rib and the palpable curved border of the ilium after a normal expiration. HC was also measured at the level of the greater trochanters. Blood pressure (BP) was measured by a digital sphygmomanometer (BC08; Beurer GmbH, Ulm, Germany).

Laboratory investigations

Glycemic status and lipid profile

Enzymatic colorimetric analysis was used for fasting serum concentration of glucose, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), total cholesterol (TC), and triglycerides (TG). (Pars Azmoon, Tehran, Iran). An enzyme immunoassay (EIA) kit was used to determine serum insulin level (Demeditec Diagnostics GmbH, Kiel, Germany). Homeostasis model assessment of insulin resistance (HOMA-IR) was used as an index of IR and was calculated according to the formula (33): fasting insulin (mU/mL) * fasting glucose (mg/dL)/405

Serum calcidiol and iPTH measurements

Serum 25(OH)D and iPTH were measured by EIA assay kits from Euroimmun, Medizinische Labordiagnostika AG, Germany. Subjects were categorized as vitamin D deficient, vitamin D insufficient, and vitamin D sufficient with 25(OH)D concentrations of below 20 ng/ml, 20-29 ng/ml, and 30 ng/ml and above respectively (34-37).

2.3. DNA Extraction and Genotyping

In order to extract genomic DNA from samples of whole blood, we used PrimePrep Genomic DNA isolation kit (GeNet Bio, Daejeon, South Korea) based on the manufacturer's instructions. For VDR FokI

polymorphism the forward primer was 5'- GTCAAAGTCTCCAGGGTCAG -3', and the reverse primer was 5'- GCCTGCTTGCTGTTCTTAC -3'. Genotyping was conducted by Applied Biosystems genetic analyzer through high-resolution melting (HRM) analysis using StepOnePlus™ (Applied Biosystems, Foster City, USA). The PCR reactions were carried out in a final volume of 20 µL using the 5x Hot FIREPol HRM Mix (HRM PCR buffer, HotStarTaq Plus DNA Polymerase, nucleotides and EvaGreen dye), 0.3 nM of forward and reverse primers each (final concentration) and 30 ng DNA under the following conditions: the initial denaturation-activation phase performed for 15 min at 95°C; then, in a 40-cycle program the reaction chamber was heated to 95°C for 15 seconds allowing for DNA denaturation, followed by the reaction temperature be lowered down to 61°C for 20 seconds allowing for annealing, and finally, elongation at 72°C for 20 seconds. The HRM assay began at 60°C rising 0.1°C until reaching 95°C. Normalized and temperature-shifted melting curves from HRM, suggestive of SNP, were distinguished, and genotyping results from the samples were confirmed by direct Sanger sequencing.

Statistical analyses

SPSS software was used for statistical analysis (IBM SPSS Statistics version 23). Continuous data were described by mean and standard deviation (SD) and categorical data were reported as frequencies. In order to determine between-group differences, independent samples *t* test, Mann–Whitney U test, or χ^2 tests were used when appropriate. Means were compared among different polymorphism groups using ANOVA or Kruskal-Wallis test. In order to quantify the associations between VDR FokI polymorphism and risk of NMSC, we fitted logistic regression models to determine the odds ratios (OR) and their corresponding 95% confidence intervals (CI). A goodness-of-fit chi-squared test was performed for finding the Hardy-Weinberg equilibrium (HWE) to show how the observed genotype frequencies are significantly different from the expected frequencies among controls. A two-tailed value of less than 0.05 was considered statistically significant.

Results

Characteristics of the study participants

Overall, 145 participants including 73 NMSC patients and 72 healthy controls were enrolled (Table 1). The mean age was 56±9 and 58±8 years for cases and controls respectively. Men had greater proportion in both study groups. Compared with the healthy controls, NMSC subjects were significantly less educated (p=0.006). BP (p=0.001), WC (p=0.016), HC (p=0.02), and percentage of visceral fat (p=0.008) were significantly greater in cases than in controls. The two groups were not statistically different in BMI (p=0.8)

Despite the fact that the duration of sun exposure (including occupational exposure) was significantly longer in cases than in controls (p<0.001), concentration of circulating 25(OH)D and distribution of vitamin D status were not statistically different between the two groups (Table 2). Fasting serum glucose level was not statistically different between cases and controls but insulin serum concentrations and

HOMA-IR were both higher in NMSC group compared to the controls. Results did not suggest any significant differences between the two groups for other variables.

Our analyses revealed that the frequencies of VDR variants in our study population were in HW equilibrium (χ^2value = 5.094, p=0.07 for control). The genotypes of FF, Ff and ff were 57%, 26%, 16% in NMSC cases and 68.1%, 23%, 8% in healthy controls, respectively. The VDR FokI polymorphism-NMSC risk association is demonstrated in Table 3. Logistic regression analysis did not show any significantly increased risk of NMSC due to f allele, as compared with FF.

When we compared different variables among VDR FokI variants, serum insulin level and HOMA-IR score were significantly higher in Ff and ff compared to the FF genotype (Table 4). Waist to hip ratio (WHR) values were greater in those with ff variant compared to those with Ff and FF variants (p=0.032). There was no significant between-FokI-variant difference in other variables.

Discussion

We found a significant association between cumulative (including occupational) sunlight exposure and increased risk of NMSC. However, no significant difference in circulating concentrations of calcidiol (25(OH)D) between NMSC subjects and their healthy counterparts was detected. A great body of evidence indicates that chronic sunlight exposure induces most non-melanoma skin cancers (38). It is roughly estimated that an individual receives 25% of their lifetime UV exposure before the age of 18 (39). Despite the potential role of sun exposure in skin cancer development and also the potential protective effect of vitamin D against various malignancies, it is still the matter of debate that how much sunlight is needed to provide adequate levels of circulating calcidiol without exerting carcinogenicity. Results from the studies that assessed the association between circulating calcidiol and NMSC are conflicting. Some case-control studies indicated an association between higher prediagnostic concentrations of circulating calcidiol and an increased risk of BCC development (31, 32). Along the same line of evidence, some prospective cohort studies reported an increased risk for developing non-melanoma and melanoma skin cancers with increasing concentrations of serum 25(OH)D (40, 41). In contrast, in a case-control study from Iran, vitamin D deficiency was highly prevalent in both BCC patients and healthy individuals (42). One reason for such discrepancies might be the very high prevalence of suboptimal serum calcidiol concentrations in Iran (43-46), which may veil any possible effect of vitamin D status on NMSC risk.

Our results did not demonstrate any significant association between VDR FokI polymorphisms and NMSC risk. Similarly, a case-control analysis nested in the Nurses' Health Study cohort did not find a significant association between FokI ff genotype and skin cancers whereas BsmI BB variant was shown to be associated with increased risk of SCC (47). In contrast with this report, a meta-analysis suggested a potential role for polymorphisms of VDR FokI and BsmI in relation to skin cancer risks including malignant melanoma and NMSC (30). Overall, previous research on the association between VDR variants and NMSC risk have generated controversial results which might be explained by genetic variations in studied populations and types of collected data.

Possible interplay between VDR variants and vitamin D status has been examined in many studies (20, 48). We found no significant difference in circulating calcidiol concentrations among VDR FokI variants. But interestingly, Fok-I Ff and ff SNPs were related to increased IR in the whole study population. Furthermore, these genotypes were related to higher BP, BMI, WC, TC, and LDL-C in both controls and NMSC cases.

There are earlier reports on the association of VDR FokI polymorphism with anthropometric and biochemical criteria of metabolic syndrome (MetS), as in a study by Filus et al. (17), VDR BsmI polymorphism was associated with BMI whereas FokI VDR variant was related to insulin sensitivity and serum HDL-C among men. In a case-control study, FokI VDR had significant association with the components of lipid panel, circulating calcidiol, and plasma levels of interleukin-6 in patients without MetS. Moreover, FokI VDR was associated with HOMA-IR, serum insulin, circulating calcidiol, and plasma levels of interleukin-6 as well as WC and BMI in the group with MetS (16). These findings are largely in accordance with those of our study. VDR gene is not the foremost determinant of 25(OH)D serum concentrations, as stated by genome-wide association (GWA) experiments. Notwithstanding, the evidence is indicative of the potential role of VDR gene in the pleiotropic functions of $1,25(OH)_2D_3$ and in insulin secretion (49). The role of increased IR in development of NMSCs needs to be clarified by further studies.

To the best of our knowledge, this is the first study to assess the possible association between Fok-I VDR gene polymorphism and IR in NMSC patients. Since IR and type 2 diabetes are caused by a set of complex interplays between genetic and lifestyle characteristics, large-scale, population-based studies are essential to further explore the relationship between this SNP and IR and its association with skin cancer development.

Some limitations of this study are acknowledged. Participants in a hospital-based case–control study may not be representative of the whole population. Secondly, comparison of the results from different studies for association between sunlight exposure and skin cancer could be very difficult because of the various methods used to estimate sunlight exposure. Case–control studies are prone to recall bias because those in the case group have the tendency to recall past exposures more accurately than controls. Moreover, self-reporting of sunlight exposures in the past may cause measurement error due to difficulty in recalling sun exposure habits. Like some other studies, some important NMSC risk factors, including family history and skin type of white subjects were not evaluated. Our data on vitamin D status were limited to a single measurement of 25(OH)D in the blood sample obtained at the enrollment time and this measurement does not necessarily reflect vitamin D status of the subjects in critical periods of life mainly childhood and young adult. Future well-designed prospective studies are to be performed to overcome the aforementioned limitations.

Conclusion

Current study indicates an interaction between VDR FokI polymorphism and measures of IR and circulating 25(OH)D. Results of this study suggests that there is an association between FokI VDR

polymorphisms and IR, which might be regarded as a genetic determinant for developing MetS in NMSC patients. Further studies involving large population and GWA studies are essential to determine the direct effect of this polymorphism on IR.

Declarations

Acknowledgement

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Conflict of interest

The authors declare that they have no conflict of interest.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Abbreviations Used

BCC: Basal cell carcinoma

BMI: Body Mass Index

DBP: Diastolic blood pressure

EIA: Enzyme immunoassay

HWE: Hardy-Weinberg equilibrium

HC: Hip circumference

HRM: high-resolution melting

HOMA-IR: Homeostasis model assessment of insulin resistance

25(OH)D: 25-hydroxycalciferol

IU: International unit

MetS: Metabolic syndrome

NMSC: Non-melanoma skin cancer

SBP: Systolic blood pressure

SCC: Squamous cell carcinoma

UV: Ultraviolet

VDD: Vitamin D deficiency

VDR: Vitamin D receptor

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Tables

Table 1. Comparison of age, gender, duration of sun exposure and certain anthropometric data of NMSC subjects and healthy controls

characteristic	NMSC (n=73)	Controls (n=72)	P value
Age (years), Mean ± SD	56±9	58±8	0.36
Sex			
male	50 (68.5%)	43 (59.7%)	0.30
female	23 (31.5%)	29 (40.3%)	
Sun exposure			
Negligible	8 (11.0%)	8 (11.1%)	
10-60 min	13 (17.8%)	38 (52.8%)	<0.001
60 min to 2 h	8 (11.0%)	9 (12.5%)	
> 2 h	44 (60.3%)	17 (23.6%)	
BMI (kg /m2)	28.04±4.3	27.88±3.8	0.80
Waist Circumference (cm)	101±10	97±9	0.016
Hip Circumference (cm)	105.4±7	102.6±6	0.02
WHR	0.95±0.05	0.94±0.05	0.14
Body fat (%)			
Truncal fat	35.7±10	34.8±8	0.53
Visceral fat	14.3±5	12.2±4	0.008
DBP (mm Hg)	13.1±2.0	12.1±1.6	0.001
SBP (mm Hg)	8.3±2	7.3±1	<0.001

BMI: body mass index; DBP: diastolic blood pressure; SBP: systolic blood pressure; WHR: waist to hip ratio

Table 2. Comparison of vitamin D, glycemic and lipidemic status between NMSC patients and healthy controls

Variable	Cases (n1=73)	Controls (n2=72)	p-value
25(OH)D (ng/mL)	30±15	29±15	0.78
Deficient (<20ng/mL), n(%)	20(27.4)	22(30.6)	
Insufficient (20-29.9 ng/mL), n(%)	23(31.5)	21(29.2)	0.9
Sufficient (>30ng/mL), n(%)	30(41.1)	29(40.3)	
iPTH (pg/mL)	46.0±20	40.5±23	0.14
Fasting serum glucose (mg/dL)	97.7±18	94.7±34	0.52
Insulin(µIU/mL)	14.8±9	12.2±6	0.048
HOMA-IR	3±2	2±1	0.037
Triglyceride(mg/dL)	129.8±75	133.3±68	0.77
HDL(mg/dL)	49.2±9	48.8±10	0.83
LDL(mg/dL)	106.3±27	105.0±27	0.78
Total cholesterol(mg/dL)	180.6±34	179.8±35	0.9

HOMA-IR: homeostasis model assessment of insulin resistance; iPTH: Intact parathyroid hormone

Table 3. Comparison of distribution of different VDR Fok-I SNPs in NMSC patients and healthy controls

Fok-I SNP	Cases (n1=73)	Controls (n2=72)	OR	95% CI	p value
FF	42(57%)	49(68%)	-		
Ff	19(26%)	17(23%)	2.33	0.806-6.75	0.12
ff	12(16%)	6(8%)	1.30	0.602-2.82	0.50
Ff+ff	31(42%)	23(31%)	1.57		0.19

Table 4. Comparison of anthropometric, blood pressure and biochemical measures among VDR FokI genotypes

Variable	Genotype (n=145)				p valuea	p valueb
	FF	**Ff**	**ff**	**Ff+ff**		
Waist(cm)	98.2±10	98.8±9	103.3±9	100.3+9	0.13	0.21
Hip (cm)	103.6±7	104.4±6	105.2±6	104.7+6	0.65	0.39
WHR	0.94±0.05	0.94±0.05	0.98±0.06	0.95+0.06	0.03	0.21
Truncal fat (%)	34.9±9	34.8±8	38.2±8	35.9+8	0.35	0.25
Visceral fat (%)	12.9±4	14.0±4	13.7±4	13.9+4	0.50	0.51
BMI (kg/m2)	27.6±4	28.3±3	28.8±3	28.4+3	0.48	0.26
DBP (mmHg)	12.5±1.9	13.0±1.8	12.2±1.8	12.7+1.9	0.32	0.52
SBP (mmHg)	7.8±1.90	7.9±1.28	7.3±1.01	7.7+1.2	0.40	0.60
25(OH)D (ng/mL)	30.6±16	28.0±14	30.1±15	28.7+14	0.69	0.46
iPTH (pg/mL)	41.3±20	48.3±24	42.7±25	46.4+24	0.29	0.18
FSG (mg/dL)	95.5±28	99.8±31	92.0±12	97.4±26	0.60	0.70
insulin (µIU/mL)	12.0±6	16.5±9	15.0 ±9	16.0±9.2	0.01	0.004
HOMA-IR	2±2	4±2	3±2	3±2	0.04	0.02
Triglyceride(mg/dL)	128.1±70	130.6±69	154.6±84	137.8±74	0.41	0.43
Total Cholesterol(mg/dL)	179.5±34	177.5±37	189.8±33	181.3±36	0.48	0.77
HDL-C (mg/dL)	49.6±10	46.9±9	50.2±9	47.9±9	0.33	0.32
LDL-C (mg/dL)	104.9±26	106.1±31	108.8±23	106.9±28	0.87	0.68

a p values stands for difference between VDR FokI genotypes (FF, Ff, ff).

b p values stands for difference between VDR FokI FF and Ff+ff genotypes.