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Research Article

Vitamin D Levels and Immune System Cells and Hematology in Saudi and Non-Saudi Males

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Abstract

Vitamin D has many important physiological functions in the body and is essential for the immune system. Vitamin D deficiency is a major widespread global public health issue. The aim of this study was to determine the relationship between serum vitamin D levels and the counts of immune system cells and other hematological parameters in healthy Saudi and non-Saudi males and the differences between the two groups of subjects for the parameters. Fifty-four Saudi and 41 non-Saudi male subjects, with an age range of 35-45 years, were randomly chosen. Each group of subjects was divided into insufficient (levels at 30-70 nmol/l), deficient (levels < 30 nmol/l), and normal (control) (levels > 70 nmol/l) vitamin D3 (25-OH) levels. Results showed that RBC counts for Saudis were significantly higher for lower vitamin D levels, while for non-Saudis there was no dependence on vitamin D levels. Neutrophil counts for both Saudis and non-Saudis were lower for lower vitamin D levels. Saudis had lower RBC counts compared with non-Saudis for the normal and insufficient groups. On the other hand, neutrophil counts were not different between Saudis and non-Saudis. In conclusion, low levels of vitamin D (insufficient and deficient) did not lead to major changes in the hematology of both groups of subjects and the existent changes were alike in both groups. Thus, it may be concluded that the subject's immune system was not affected considerably by the sub-normal levels of vitamin D and that both Saudis and non-Saudis were affected in the same way.

Keywords: Vitamin D; Immunity; Immune system cells; Hematology; Saudi males; Non-Saudi males

Introduction

Vitamin D is a fat-soluble vitamin that is essential in small amounts for human health and proper function in the body. Vitamin D deficiency is a major widespread global public health issue. It is estimated that around 1 billion people suffer from hypovitaminosis D [1]. Hypovitaminosis D is very common among Saudi Arabian adults where the prevalence was 17.7-100% for all healthy and non-healthy age groups from the year 1984 to 2015 [2-8]. It is estimated that in 2015 the prevalence of hypovitaminosis D was 40.6% for men and about 63% for women in Saudi Arabia [8]. Vitamin D has many important physiological functions in the body, such as maintaining normal serum calcium and phosphate concentrations and enabling proper mineralization and growth of bones for strong healthy bones. Vitamin D in the human body is found in two forms, termed D2 and D3, which differ slightly in their structures and source. Vitamin D3, commonly known as cholecalciferol, is derived from the diet and is produced in the skin upon sun exposure, while vitamin D2 is termed ergocalciferol and is found in plants. Vitamin D deficiency leads to many conditions and diseases, with some being skeletal and others non-skeletal, such as increased fracture risk, tooth loss, rickets in children, osteomalacia and osteoporosis in adults [9-12], body aches, fatigue, and myopathy [13] In addition, hypovitaminosis D is associated with an increased risk for infectious diseases [14], diabetes mellitus [15], metabolic syndrome [16], dementia [17], cardiovascular diseases [18], and certain types of cancers [19-21], rheumatoid arthritis [22], multiple sclerosis [23], depression [24], schizophrenia [25], and obesity [16-27]. On the other hand, some diseases lead to hypovitaminosis D, such as liver disease, renal failure, and any condition or disease that involves or effects organs involved in vitamin D metabolism [28-30]. Hypovitaminosis D is usually termed as insufficiency or deficiency with the deficient levels being lower than the insufficient levels. The ranges and cutoff points for these two categories vary widely and none are approved worldwide. Worldwide, vitamin D levels [31-33] used for insufficient vitamin D vary from 30 up to 75nmol/L while deficient levels range from < 20 to < 50 nmol/L. Likewise, the vitamin D levels considered normal or healthy vary from > 70 to > 75 nmol/L. Finally, hypertoxicity of vitamin D is considered to be around 250 nmol/L. Worldwide, around 50% of the world population suffer from vitamin D insufficiency [34], while around 37% suffer from deficiency [35]. Lower than normal levels of vitamin D are mainly found in the elderly and is more prevalent in females compared to males [1,11].

The major causes of hypovitaminosis D are a diet lacking in vitamin D, low or no exposure to the sunlight, and problems with absorbing vitamin D in the body [36]. Other factors implicated in low vitamin D levels are such as more melanin in the skin (darker skin), overweight and obesity, older age, remaining indoors most of the time, using sunscreen wearing clothing that covers most of the body, low intake of vitamin D-containing foods, and some disease that lead to low vitamin D levels [36-39]. Sunny countries are not immune from hypovitaminosis and in fact many such countries have a high prevalence of hypovitaminosis [40,41]. It has been observed that the prevalence of hypovitaminosis D among Saudis has been increasing for the last four decades [4-43]. The proposed reasons for this are the major changes in the lifestyle for Saudis towards a more relaxed sedentary lifestyle and the greater availability of different types of foods including Western-type foods leading to more overweight and obesity and their subsequent related diseases and conditions [2]. It is known that increased body weight leads to lower vitamin D levels since vitamin D is sequestered in body fat due to it being a fat-soluble vitamin [44]. The prevalence rates of hypovitaminosis D vary widely between countries [40-46] and between different ethnic groups [40,41] with white Caucasians having lower vitamin D levels than nonwhite individuals. Some studies found Saudis have a higher prevalence of hypovitaminosis D compared to no Saudis living in Saudi Arabia and even in the same areas [47]. An explanation for this is the genetic differences between different ethnic groups [48-51] and the genetic influence on vitamin D levels in the body [49,51]. Vitamin D is important for many systems and organs in the body as suggested by the presence of the vitamin D receptor (VDR) on many cells of the body including cells of the bone marrow, colon, breast, brain and cells of the immune system including lymphocytes [52]. Thus, vitamin D has an important role in the immune system and response [53,54]. Vitamin D has been shown to enhance both the innate and acquired immune systems through different mechanisms [55-59]. Most cells of the immune system, except B lymphocytes, have the VDR on their membrane [60], making vitamin D essential for a strong immune response and explaining the fact that people with low vitamin D levels are more prone to infections and to be infected by infectious diseases [61]. The immune system is one of the most important systems in the body. Its main function is to protect against infections while at the same time it is sensitive to changes and conditions of the body [62]. The two types of the immune response mediate their actions through cells, tissues and molecules. The major white blood cells of the innate immune response are the neutrophils, monocytes, eosinophils and basophils, while for the acquired immune response it is the lymphocytes along with other cells and components of the immune system. Vitamin D deficiency is linked to an increased risk for serious diseases and has numerous effects on cells within the immune system. This study determined vitamin D levels and the counts of white blood cells and other hematological parameters (platelets and red blood cell counts, and hemoglobin concentration) in healthy male adult Saudis and non-Saudis. This was done to determine the relationship between the vitamin D levels and the state of the immune system cells and other hematological parameters and to ascertain if there are differences between Saudis and non-Saudis in these effects. This is the first study to determine the differences in the vitamin D levels and the differential complete blood count (CBC) between both Saudi and non-Saudi subjects living in Saudi Arabia (Figure 1).

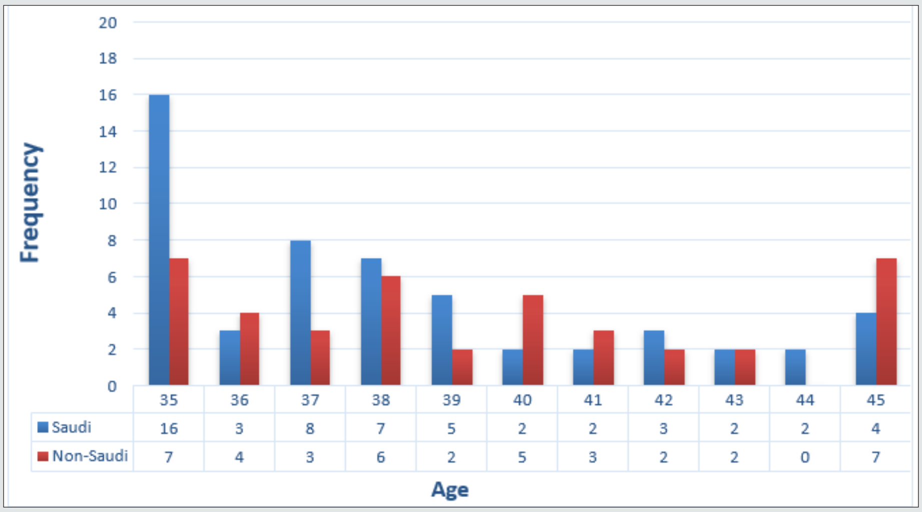

Figure 1: Frequency distribution of ages of vitamin D levels in healthy Saudi and Non-Saudi males.

Materials and Methods

Subjects and categorizations

The subjects were 95 randomly chosen male blood donors at King Abdulaziz University, with an age range of 35-45 years and living in Jeddah, Saudi Arabia. Of the subjects, 54 were Saudis and 41 were non-Saudis. None of the subjects had any chronic, genetic, or immune-related diseases nor were they taking vitamin D supplements. As for the presence of other diseases, one subject had eczema and was taking medications for it, and 5 subjects had asthma, hey fear, or dust allergies but only one of these subjects was taking medications for his condition. The only other subjects taking medications were two subjects taking medications for acid reflux, one taking iron supplements and one taking prostate medications. This study was reviewed and approved by the Unit of Biomedical Ethics Research Committee at King Abdulaziz University, Jeddah, Saudi Arabia. All subjects gave written informed consent and filled a questionnaire on health and lifestyle factors that may influence the parameters. Subjects were categorized as Saudi and non-Saudi, and subsequently each group of subjects was divided into three groups based on the subject's vitamin D3 (25-OH) level as follows: insufficient (levels equal 30-70 nmol/l), deficient (levels less than 30 nmol/l), and normal vitamin D levels (control) (levels more than 70 nmol/l).

Blood Collection

Venous blood samples were collected from each subject into ethylenediaminetetraacetic acid (EDTA) vacutainer tubes for the CBC analysis. Whole blood was collected into gel separator vacutainer tubes for the determination of vitamin D. Serum samples were obtained by centrifugation at 5,000 RPM for 10 minutes. Serum and plasma samples was stored at -40 °C until use.

Determination of vitamin D concentrations

The serum concentrations of vitamin D3 were measured on a Cobas e 601 Systems instrument (Modular analytics E170, Roche Company, Frankfurt, Germany) using the Reagent Rackpack and the pretreatment reagents (VITD-T) (Roche Diagnostics GmbH Company, Mannheim, Germany).

Determination of the differential complete blood counts

The CBC for each blood sample was done on an XN-9000 Hematology System instrument (Sysmex Europe GmbH, Kobe, Japan), using Cellpack, Sulfolyzer, Fluorocell and Lysercell Reagents (Sysmex Corporation, Kobe, Japan).

Statistical analysis

Data were statistically analyzed by using the Megastat statistical program (version 10.3) and the range (minimum and maximum) mean, standard deviation (± SD), and the standard error of the mean (± SE) were determined for all results. After testing for the homogeneity and normality of the parameters, the ANOVA one-way test was used to test for the presence of significant differences between the different groups of subjects for the normally distributed parameters. As for the homogeneous non-normally distributed parameters, the Kruskal-Walli's test was used to test for the significance in the differences between the groups. The T-test was used to test for the presence of significant differences for the normally distributed parameters between the normal and deficient groups. Additionally, the Mann-Whitney U test was used to test for the significance in the comparison between the normal and deficient groups for the non-normally distributed parameters. Subsequently, for the post hoc comparisons for the differences between the groups, the Mann-Whitney U test was used for the age and neutrophil counts for non-Saudis, while the T-test was used for the vitamin D levels for non-Saudi subjects. The resulting P values demonstrate significance or lack thereof as follows: $P > 0.05$ is a non-significant (NS) difference, $0.01 \le P \le 0.05$ is a significant (S) difference, $P < 0.01$ is a highly significant (HS) difference.

Results

Subjects' characteristics, categorizations, and lifestyle questionnaire

All subjects filled a lifestyle questionnaire to find any lifestyle differences within the groups and between Saudis and non-Saudi (Table 1). The number of Saudi subjects (Table 2) in the vitamin D levels groups were three (5.56%) for the normal, 18 (33.33%) for the insufficient, and 33 (61.11%) for the deficient vitamin D levels. The normal groups were not included in the comparisons of the parameters between the different vitamin D level groups due to the very low number of subjects. As for the non-Saudis groups, the number of subjects for the normal group was five (12.20%), for the insufficient they were 23 (56.10%), and, finally, for the deficient they were 13 (31.71%). In addition, another categorization was done where the normal and insufficient subjects were combined into one group in addition to the deficient group. For Saudis the normal/insufficient group had 21 (22.11%) subjects, while for non-Saudis the combined group had 28 (29.47) subjects. The other group (deficient) remains unchanged. Both groups of subjects (Saudi and non-Saudi) had an age range of 35-45 years. The mean age, weight, height, and heart rate for the Saudi and non-Saudi subjects are shown in Tables 3-5. These parameters were compared between the insufficient and deficient Saudi subjects using the T-test (Tables 3) and no significant differences were found. The normal Saudi subjects were not compared with the other vitamin D groups since the number of subjects (3) is too low. The comparison between the mean ages for the normal, insufficient and deficient groups for the non-Saudi subjects, using the Kruskal-Wallis test (Table 3), showed a significant difference. As for the post hoc comparisons between groups, using the Mann-Whitney U test (Table 4), the mean age for the normal group was significantly higher than for the deficient groups while there were no significant differences for the other group comparisons. The mean weight, height and heart rate for the three vitamin D groups for non-Saudis, using the one-way ANOVA test (Table 3), were not significantly different. The normal and insufficient groups were combined into one group termed normal/insufficient group. Comparing between the normal/insufficient and deficient groups, using the T-test (Table 5), showed no significant differences for the mean age for Saudis and the mean weight, height and heart rate for Saudis and non-Saudis, separately. The mean age for non-Saudis was significantly higher for the normal/insufficient group compared to the deficient group, using the T-test (Table 5). Statistical comparisons between the Saudi and non-Saudi subjects for the normal/insufficient, insufficient and deficient groups are shown in Table 6. Comparing the same groups between Saudi and non-Saudi subjects, the mean age for the non-Saudis was significantly higher between the normal/insufficient groups, using the T-test, and the insufficient groups, using the Mann-Whitney U Test, while it was not significantly different between the deficient groups, using the T-test. As for the mean weight, height and heart rate, using the T-test, there were no significant differences between the Saudis and non-Saudis for each of the three vitamin D groups.

Table 1: Lifestyle questionnaire within the groups and between Saudis and non-Saudi.

Factor	Normal vitamin D				Insufficient vitamin D				Deficient vitamin D				Total			
	S (n = 3)		NS (n = 5)		S (n = 18)		NS (n = 23)		S (n = 33)		NS (n = 13)		S (n = 54)		NS (n = 41)	
	Freq.	%	Freq.	%	Freq.	%	Freq.	%	Freq.	%	Freq.	%	Freq.	%	Freq.	%
Education level																
Less than high school	1	33.33	1	20	2	11.11	7	30.43	5	15.15	2	15.38	8	14.81	10	24.39
High school	0	0	4	80	5	27.78	5	21.74	9	27.27	6	46.15	14	25.93	15	36.59
Diploma	0	0	0	0	2	11.11	1	4.35	2	6.06	0	0	4	7.41	1	2.44
Bachelor	1	33.33	0	0	7	38.89	10	43.48	17	51.52	5	38.46	25	46.3	15	36.59
Master	1	33.33	0	0	2	11.11	0	0	0	0	0	0	3	5.56	0	0
Ph.D.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Affliction with diseases																
Yes	0	0	0	0	0	0	1	4.35	0	0	0	0	0	0	1	2.44
No	3	100	5	100	18	100	22	95.65	33	100	13	100	54	100	40	97.56
Any immune, genetic diseases																
Yes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
No	3	100	5	100	18	100	23	100	33	100	13	100	54	100	41	100
On any medications																
Yes	0	0	0	0	0	0	1	4.35	2	6.06	1	7.69	2	3.7	2	4.88
No	3	100	5	100	18	100	22	95.65	31	93.94	12	92.31	52	96.3	39	95.12
Allergies																
Yes	0	0	0	0	3	16.67	1	4.35	2	6.06	1	7..69	5	9.26	2	4.88
No	3	100	5	100	15	83.33	22	95.65	31	93.94	12	92.31	49	90.74	39	95.12
Allergies Medications																
Yes	0	0	0	0	1	5.56	0	0	1	3.03	1	7..69	2	3.7	1	2.44
No	3	100	5	100	17	94.44	23	100	32	96.97	12	92.31	52	96.3	40	97.56
Deficient vitamin D																
Yes	2	66.67	0	0	5	27.78	0	0	0	0	2	15.38	7	12.96	2	4.88
No	0	0	2	40	5	27.78	12	52.17	11	33.33	1	7.69	16	29.63	15	36.58
Don't no	1	33.33	3	60	8	44.44	11	47.83	22	66.67	10	76.92	31	57.41	24	58.54
Medication for vitamin D or any vitamins																
Yes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
No	3	100	5	100	18	100	23	100	33	100	13	100	54	100	41	100
Sun exposure between 10 am to 3 pm																
Yes	2	66.67	5	100	15	83.33	22	95.65	28	84.85	10	76.92	45	83.33	37	90.24
No	1	33.33	0	0	3	16.67	1	4.35	5	15.15	3	23.08	9	16.67	4	9.76

Frequency of sun exposure																
Daily	2	66.67	4	80	10	5.56	18	78.26	24	72.73	7	53.85	36	66.67	29	70.73
Twice or third daily	1	33.33	1	20	7	38.89	5	21.74	6	18.18	3	23.08	14	25.93	9	21.95
Once weekly	0	0	0	0	1	5.56	0	0	3	9.09	3	23.08	4	7.41	3	7.32
Foods's rich in vitamin D																
Often	2	66.67	2	40	6	33.33	9	39.13	20	60.61	4	30.77	28	51.85	15	36.59
Few	1	33.33	3	60	9	50	12	52.17	12	36.36	8	61.54	22	40.74	23	56.1
Rare	0	0	0	0	3	16.67	2	8.7	1	3.03	1	7.69	4	7.41	3	7.317
Never	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Frequency of vitamin D rich foods																
Daily	1	33.33	1	20	4	22.22	1	4.35	10	30.3	4	30.77	15	27.78	6	14.63
Tow or third daily	2	66.67	3	60	8	44.44	20	86.96	19	57.58	5	38.46	29	53.7	28	68.29
Once weekly	0	0	1	20	6	33.33	2	8.7	4	12.12	4	30.77	10	18.52	7	17.07
Foods's rich in immune enhancers																
Few	1	33.33	3	60	10	55.56	10	43.48	15	45.45	6	46.15	26	48.15	19	46.34
Little	2	66.67	2	40	7	38.89	11	47.83	18	54.55	7	53.85	27	50	20	48.78
Rare	0	0	0	0	1	5.56	1	4.35	0	0	0	0	1	1.85	1	2.44
Never	0	0	0	0	0	0	1	4.35	0	0	0	0	0	0	1	2.44
Frequency of exercise																
Daily	1	33.33	0	0	7	38.89	3	13.04	7	21.21	3	23.08	15	27.78	6	14.63
Weekly	1	33.33	0	0	5	27.78	4	17.39	6	18.18	0	0	12	22.22	4	9.76
Monthly	0	0	0	0	0	0	0	0	1	3.03	0	0	1	1.85	0	0
Rare	1	33.33	0	0	2	11.11	5	21.74	14	42.42	3	23.08	17	31.48	8	19.51
Never	0	0	5	100	4	22.22	11	47.83	5	15.15	7	53.85	9	16.67	23	56.1
Tea and coffee drinking																
Tea only	0	0	2	40	4	22.22	6	26.09	6	18.18	5	38.46	10	18.52	13	31.71
Coffee only	2	66.67	0	0	5	27.78	6	26.09	5	15.15	2	15.38	12	22.22	8	19.51
Tea and coffee	1	33.33	2	40	8	44.44	9	39.13	21	63.64	5	38.46	30	55.56	16	39.02
None	0	0	1	20	1	5.56	2	8.7	1	3.03	1	7.69	2	3.7	4	9.76
First-hand Smokers																
Yes	1	33.33	2	40	4	22.22	8	34.78	19	57.58	6	46.15	24	44.44	16	39.02
No	2	66.67	3	60	14	77.78	15	65.22	14	42.42	7	53.85	30	55.56	25	60.98
Exposure to second-hand smoke																
At home	0	0	0	0	0	0	4	17.39	0	0	0	0	0	0	4	9.76
At work	1	33.33	4	80	8	44.44	6	26.09	20	60.61	7	53.85	29	53.7	17	41.46
No	2	66.67	1	20	10	55.56	13	56.52	13	39.39	6	46.15	25	46.3	20	48.78
Frequency of exposure to second-hand Smoke																
Daily	1	33.33	3	60	5	27.78	8	34.78	11	33.33	3	23.08	17	31.48	14	34.15
Weekly	0	0	1	20	3	16.67	1	4.35	9	27.27	4	30.77	12	22.22	6	14.63
Never	2	66.67	1	20	10	55.56	14	60.87	13	39.39	6	46.15	25	46.3	21	51.22
Hours of sleep																
Day time	0	0	0	0	0	0	0	0	2	6.06	1	7.69	2	3.7	1	2.44
Nighttime	3	100	5	100	17	94.44	19	82.61	27	81.82	12	92.31	47	87.04	36	87.8
Both	0	0	0	0	1	5.56	4	17.39	4	12.12	0	0	5	9.26	4	9.76

S: Saudi, NS: Non-Saudi

Table 2: The number and percent of subjects in each group of vitamin D level among Saudis and non-Saudis.

Vitamin D Level Group	Saudi		Non-Saudi	
	N	%	N	%
Normal	3	5.56	5	12.2
Insufficient	18	33.33	23	56.1
Deficient	33	61.11	13	31.71
Total	54	100	41	100

Table 3: Statistical analysis of the mean age, weight, height, and heart rate for Saudi and non-Saudi subjects and the P-values for the differences between the vitamin D levels groups.

Parameter	Saudi							Non-Saudi						
	Normal		Insufficient		Deficient			Normal		Insufficient		Deficient		
	Mean	±SD	Mean	±S D	Mean	±SD	P-value	Mean	±SD	Mean	±SD	Mean	±SD	P-value
Age^{T,K} (Years)	38	3	38	3	39	3	0.332^{NS}	42	3	40	3	38	3	0.037^{S}
Weight^{T,O} (Kg)	85	29	88	17	95	19	0.210^{NS}	86	13	90	14	94	15	0.490^{NS}
Height^{T,O} (cm^2)	169	12	169	5	169	6	0.693NS	168	6	170	7	169	5	0.746^{NS}
Heart rate^{T,O} (BPM)	71	14	78	11	81	11	0.362^{NS}	78	11	80	12	82	9	0.761^{NS}

T: T-test, O: One way ANOVA test, K: Kruskal-Walli's test were used for the significance testing

NS: Not significant (P > 0.05), S: Significant (P ≤ 0.05)

Table 4: Post hoc statistical analysis for the statistical differences between the groups in Table 3 and 7.

Parameter	Statistical Test	Group 1	Group 2	Mean Difference (1-2)	±SE	P-value
Age	Mann-Whitney U	Normal	Insufficient	2	1.66	0.167^{NS}
			Deficient	4	1.67	0.026^{S}
		Insufficient	Deficient	2	1.14	0.067^{NS}
Vitamin D	T-test	Normal	Insufficient	40.74	4.92	0.000^{HS}
			Deficient	63.66	4.54	0.000^{HS}
		Insufficient	Deficient	22.92	3.07	0.000^{HS}
Neutrophils	Mann-Whitney U	Normal	Insufficient	-0.51	0.76	0.293^{NS}
			Deficient	0.86	0.52	0.882^{NS}
		Insufficient	Deficient	1.37	0.41	0.001^{HS}

NS: Not significant (P > 0.05), HS: High significant (P < 0.01), S: Significant (P ≤ 0.05)

Table 5: Statistical analysis of mean age, weight, height, and heart rate for Saudi and non-Saudi subjects and the P-values for the differences between the two groups of the vitamin D levels.

Parameters	Saudi					Non-Saudi				
	Normal/Insufficient		Deficient			Normal/Insufficient		Deficient		
	Mean	±SD	Mean	±SD	P-value	Mean	±SD	Mean	±SD	P-value
Age (Years)	38	3	39	3	0.355^{NS}	40	3	38	3	0.032^{S}
Weight (Kg)	88	18	95	19	0.172^{NS}	90	14	94	15	0.324^{NS}
Height (cm^2)	169	6	169	6	0.733^{NS}	170	7	169	5	0.925^{NS}
Heart rate (BPM)	77	11	81	11	0.205^{NS}	80	11	82	9	0.546^{NS}

T-test was used for the significance testing

NS: Not significant (P > 0.05), S: Significant (P ≤ 0.05)

Table 6: Statistical analysis of mean age, weight, height, and heart rate for the vitamin D level groups and the P-values for the differences between Saudi and non-Saudi subjects.

Parameter	Normal/Insufficient					Insufficient					Deficient				
	Saudi		Non-Saudi			Saudi		Non-Saudi			Saudi		Non-Saudi		
	Mean	±SD	Mean	±SD	P-value	Mean	±SD	Mean	±SD	P-value	Mean	±SD	Mean	±SD	P-value
Age^{T,M,T} (Years)	38	3	40	3	0.013^{S}	38	3	40	3	0.039^{S}	39	3	38	3	0.407^{NS}
Weight^{T} (Kg)	88	18	90	14	0.692^{NS}	88	17	90	14	0.641^{NS}	95	19	94	15	0.913^{NS}
Height^{T} (cm^{2})	169	6	170	7	0.690^{NS}	169	75	170	67	0.518^{NS}	169	6	169	5	0.977^{NS}
Heart rate^{T} (BPM)	77	11	80	11	0.304^{NS}	78	11	80	12	0.436^{NS}	81	11	82	9	0.629^{NS}

T-test and Mann-Whitney U test were used for the significance testing

NS: Not significant (P > 0.05), S: Significant (P ≤ 0.05)

Determination of vitamin D concentrations and CBC

Statistical analysis for the two vitamin D groups for the Saudis, using the T-test (Table 7), showed a highly significantly lower mean vitamin D level for the deficient group compared to the mean level for the insufficient group. The mean vitamin D concentrations for the vitamin D groups for non-Saudis, using the ANOVA one-way test (Table 7), showed a significant difference. The between group comparisons, using the T-test (Table 4), showed highly significantly lower mean vitamin D levels for the insufficient and deficient groups compared with the normal vitamin D group and for the deficient group compared with the insufficient group. The other parameters that showed a significant difference between the groups were the mean RBC counts for Saudis and the mean neutrophil counts for both Saudis and non-Saudis. The mean RBC counts for the Saudi deficient group was significantly higher, using the T-test, compared to that of the insufficient. The mean neutrophil count for the deficient Saudis, using the T-test, was significantly lower compared with count for the insufficient group. The mean neutrophil counts for the non-Saudis, using the Kruskal-Wallis test, was significantly different between the three vitamin D groups. As for the post hoc comparisons, using the Mann-Whitney U test (Table 4), the mean neutrophil counts were not significantly different between both the insufficient and deficient groups compared with the normal group, while the counts for the deficient group was highly significantly lower than for the insufficient group. All other cell counts and comparisons did not show significant differences. The statistical comparisons for the parameters between the normal/insufficient and deficient groups for the Saudi and non-Saudi subjects are shown in Table 8. The mean vitamin D levels were highly significantly lower for the deficient groups for the Saudi and non-Saudi subjects, using the T-test. The mean RBC count for the Saudis deficient group was significantly higher, using the Mann-Whitney U test, compared to the normal/insufficient group mean counts. On the other hand, the mean RBC counts for the non-Saudis were not significantly different, using the T-test, between the groups. The mean neutrophil counts for the Saudis and non-Saudi, using the T-test, were highly significantly lower for each deficient group compared to the respective normal/insufficient group. All other parameters for the Saudi and non-Saudi subjects did not show any significant differences. Comparing the vitamin D concentrations and differential CBC between Saudi and non-Saudi subjects for the normal/insufficient, insufficient, and deficient groups (Table 9), there were no significant differences for all parameters except for the mean RBC counts. The mean RBC counts for the normal/insufficient and insufficient, using the Mann-Whitney U test, were significantly higher for non-Saudi subjects compared to the respective level for Saudi subjects.

Table 7: Statistical analysis of the mean hematological parameters and vitamin D concentrations for Saudi and non-Saudi subjects and the P-values for the differences between the vitamin D level groups.

Parameter	Saudi							Non-Saudi						
	Normal		Insufficient		Deficient			Normal		Insufficient		Deficient		
	Mean	±SD	Mean	±SD	Mean	±SD	P-value	Mean	±SD	Mean	±SD	Mean	±SD	P-value
Vitamin D^{T,O}	75.23	10.69	39	7.9	18.33	6	0.000^{HS}	81.81	11.81	41.07	9.61	18.15	7.27	0.000^{HS}
RBC^{M,K}	5.35	2.22	5.3	0.61	5.64	0.67	0.048^{S}	5.52	0.57	5.67	0.61	5.42	0.32	0.464^{NS}
Platelets^{T,O}	277	75	266	40	256	44	0.434^{NS}	247	29	258	60	238	47	0.581^{NS}
Hemoglobin^{T,O}	16.1	1.1	15	1	15.4	1.2	0.275^{NS}	15	1.3	15.6	0.8	15.8	0.9	0.224^{NS}
WBC^{T,K}	7.67	1.17	6.88	1.46	6.84	2.02	0.948^{NS}	6.55	1.98	7.61	3.21	6.56	1.74	0.510^{NS}
Neutrophils^{T,K}	4.24	0.58	3.16	1.47	2.25	0.2	0.000^{HS}	3.09	1.93	3.6	1.45	2.23	0.26	0.008^{HS}
Lymphocytes^{T,O}	2.66	0.77	2.79	0.54	2.69	0.87	0.649^{NS}	2.7	0.84	2.79	0.78	2.75	0.51	0.966^{NS}
Monocytes^{T,O}	0.59	0.13	0.59	0.17	0.64	0.18	0.340^{NS}	0.5	0.14	0.56	0.22	0.59	0.29	0.797^{NS}

Eosinophils^{T,O}	0.15	0.07	0.27	0.14	0.2	0.11	0.061^{NS}	0.18	0.12	0.26	0.13	0.19	0.09	0.137^{NS}
Basophil^{M,K}	0.03	0.01	0.06	0.09	0.04	0.03	0.095^{NS}	0.04	0.02	0.04	0.03	0.04	0.03	0.919^{NS}

T-test, One way ANOVA test, Kruskal-Wallis test and Mann-Whitney U test were used for the significance testing

NS: Not significant (P > 0.05), HS: High significant (P < 0.01)

Max: Maximum, Min: Minimum

Table 8: Statistical analysis for the mean hematological parameters and vitamin D concentrations for Saudi and non-Saudi subjects and the P-values for the differences between two groups of the vitamin D levels.

Parameter	Saudi					Non-Saudi				
	Normal/Insufficient		Deficient			Normal/Insufficient		Deficient		
	Mean	±SD	Mean	±SD	P-value	Mean	±SD	Mean	±SD	P-value
Vitamin D^{T}	44.1	15.29	18.33	5.93	0.000^{HS}	48.35	18.67	18.15	7.27	0.000^{HS}
RBC^{M,T}	5.31	0.57	5.64	0.68	0.042^{S}	5.64	0.59	5.42	0.21	0.222^{NS}
Platelets^{T}	268	44	256	44	0.359^{NS}	256	55	238	47	0.331^{NS}
Hemoglobin^{T}	15.19	1.03	15.4	1.22	0.514^{NS}	15.48	0.94	15.8	0.9	0.290^{NS}
WBC^{T}	6.99	1.43	6.84	2.02	0.770^{NS}	7.42	3.03	6.56	1.74	0.347^{NS}
Neutrophils^{T}	3.31	1.42	2.25	0.2	0.000^{HS}	3.51	1.52	2.23	0.26	0.005^{HS}
Lymphocytes^{T}	2.77	0.55	2.69	0.87	0.692^{NS}	2.77	0.78	2.75	0.51	0.925^{NS}
Monocytes^{T}	0.58	0.16	0.64	0.18	0.311^{NS}	0.55	0.21	0.59	0.29	0.628^{NS}
Eosinophils^{T}	0.25	0.14	0.2	0.11	0.145^{NS}	0.25	0.13	0.19	0.09	0.163^{NS}
Basophil^{T,M}	0.04	0.01	0.04	0.02	0.761^{NS}	0.04	0.03	0.04	0.03	0.899^{NS}

T-test and Mann-Whitney U test were used for the significance testing

NS: Not significant (P > 0.05), HS: High significant (P < 0.01), S: Significant (P ≤ 0.05)

Table 9: Statistical analysis for the mean hematological parameters and vitamin D concentrations for the vitamin D level groups and the P-values for the differences between Saudi and non-Saudi subjects.

Parameter	Normal/Insufficient					Insufficient					Deficient				
	Saudi		Non-Saudi			Saudi		Non-Saudi			Saudi		Non-Saudi		
	Mean	±SD	Mean	±SD	P-value	Mean	±SD	Mean	±SD	P-value	Mean	±SD	Mean	±SD	P-value
Vitamin D^{M,T,T}	44.1	15.3	48.4	18.7	0.442^{NS}	38.9	7.87	41.07	9.62	0.444^{NS}	18.3	5.93	18.15	7.27	0.929^{NS}
RBC^{T,M,T}	5.31	0.57	5.64	0.59	0.019^{S}	5.31	0.62	5.67	0.61	0.029^{S}	5.64	0.68	5.42	0.32	0.266^{NS}
PlateletsT	268	44	256	55	0.426^{NS}	266	40	258	60	0.610^{NS}	256	44	238	48	0.227^{NS}
Hemoglobin^{T}	15.2	1	15.5	0.9	0.326^{NS}	15	1	15.6	1	0.058^{NS}	15.4	1.2	16	1	0.288^{NS}
WBC^{T}	6.99	1.43	7.42	3.03	0.544^{NS}	6.88	1.46	7.61	3.21	0.371^{NS}	6.84	2.02	6.56	1.74	0.666^{NS}
Neutrophils^{T}	3.32	1.42	3.59	1.52	0.652^{NS}	3.16	1.47	3.6	1.45	0.345^{NS}	2.25	0.2	2.23	0.26	0.798^{NS}
Lymphocyte^{T}	2.77	0.55	2.77	0.78	0.999^{NS}	2.79	0.54	2.79	0.78	0.987^{NS}	2.68	0.87	2.75	0.51	0.810^{NS}
Monocytes^{T}	0.59	0.16	0.55	0.21	0.463^{NS}	0.59	0.17	0.56	0.22	0.633^{NS}	0.64	0.18	0.59	0.29	0.467^{NS}
Eosinophils^{T}	0.25	0.14	0.25	0.13	0.825^{NS}	0.27	0.14	0.26	0.13	0.789^{NS}	0.2	0.11	0.19	0.09	0.666^{NS}
Basophil^{M}	0.04	0.01	0.04	0.03	0.524^{NS}	0.06	0.09	0.04	0.03	0.454^{NS}	0.04	0.03	0.04	0.03	0.678^{NS}

T-test and Mann-Whitney U test were used for the significance testing

NS: Not significant (P > 0.05), S: Significant (P ≤ 0.05)

Discussion

There are no studies on the effects of vitamin D levels on the CBC in healthy Saudi and non-Saudi subjects living in Saudi Arabia and the differences between them. Most available studies are statistical in nature and mainly focus on lifestyle. Therefore, this study is the first to determine the CBC for both healthy Saudi and non-Saudi subjects residing in Saudi Arabia and to determine the differences between the CBC for the two groups. In addition, anthropometric measurements were taken, and a lifestyle questionnaire was used and compared between the two groups of subjects.

For the number of subjects in the groups of the study, most Saudi subjects had deficient vitamin D levels followed by the insufficient subjects. On the other hand, the non-Saudi subjects were the opposite with most subjects being insufficient followed by deficient. The Saudi and non-Saudi subjects with normal vitamin D levels, in general, had lower levels of education compared to the Saudi and non-Saudi subjects with insufficient and deficient vitamin D levels. This is contradictory to the findings of a previous study [5-65] that found a lack of education in non-Saudi subjects with low levels of vitamin D. Most subjects with lower-than-normal vitamin D levels did not know that they had low levels or thought they did not. Concerning sun exposure, most subjects (Saudi and none) were exposed daily. Most Saudis in the deficient group consumed many foods rich in vitamin D, such as milk, cooked liver, salmon, eggs, tuna, mushrooms and sardine, while the Saudis in the insufficient group and non-Saudis in both low vitamin D groups consumed few foods rich in vitamin D, and all consumed these foods 2-3 days per week. As for foods that enhance immunity, such as garlic, honey, vitamin C, green tea and vegetables (tomato, broccoli, colliflower, cabbage, and turnip), most subjects consumed them often or few. As for exercise, most of the non-Saudis with normal vitamin D levels never exercised. Most of the Saudis in the insufficient group exercised daily while the ones in the deficient group rarely exercised. As for the non-Saudis, most of them never exercised. Most subjects drank coffee and/or tea. Most of the Saudi and non-Saudi subjects were non-smokers except for the Saudi deficient subjects that were mostly smokers. Most of the Saudi and non-Saudi insufficient vitamin D level subjects were not exposed to second-hand smoke while most of the Saudi and non-Saudi deficient subjects were exposed to second-hand smoke. Subjects that were exposed to second-hand smoke were exposed to it daily and mainly at work. Most subjects slept most of their sleeping hours at night. The comparisons between the vitamin D level groups among Saudi and non-Saudi subjects separately, and for each vitamin D level group between Saudi and non-Saudi subjects for the mean age, weight, height, and heart rate, showed that only the age showed significant differences. The mean ages for the three vitamin D level groups for the non-Saudi subjects showed a significantly lower mean age for the deficient group compared with the mean age for the normal group. Similarly, the mean ages for the two vitamin D groups for non-Saudis showed a significantly lower mean age for the deficient group compared with the normal/insufficient group. As for the comparison between Saudi and non-Saudi subjects, the mean age for the non-Saudi subjects was significantly higher than for the Saudi subjects for both the normal/insufficient and insufficient groups. As for the other parameters, there were no significant differences for all comparisons. These findings agree with the findings of [65] that vitamin D prevalence was less in older people. While the findings disagree with the previous studies [3,66] that found higher ages for people with lower vitamin D levels. The non-significant difference in the weight of the subjects disagree with the previous finding [44,67] of lower vitamin D levels in people with higher body weight or obesity. The no effect of vitamin D on the heart rates of the subjects agrees with previous findings [68].

The findings of this study for the two vitamin D groups for the Saudis showed a highly significantly lower mean vitamin D level for the deficient group compared with the mean vitamin D level for the insufficient group. Likewise, the mean vitamin D levels for the insufficient and deficient groups were highly significantly lower than for the normal vitamin D group. The deficient group had highly significantly lower vitamin D levels compared to the normal/insufficient groups in Saudi and non-Saudi subjects separately. These above findings are expected since the vitamin D level groups were designed to categorize the patients and differentiate between them according to their vitamin D levels. On the other hand, no significant differences were found between Saudi and non-Saudi subjects in the mean vitamin D levels for each of the normal/insufficient, insufficient and deficient vitamin D groups. This last finding disagrees with the previous studies [47,64] that found a significant increase in vitamin D deficiency in Saudi compared to non-Saudi subjects. As for the mean RBC counts in Saudi subjects, they were significantly higher for the deficient group compared with each of the insufficient and normal/insufficient groups, while for the non-Saudi subjects there were no significant differences between the groups. As for the comparison between Saudi and non-Saudi subjects, non-Saudi subjects had significantly higher mean RBC counts compared with the non-Saudis for the normal/insufficient and insufficient groups, separately, while the deficient groups were not significantly different. Mean neutrophil counts for the Saudi deficient subjects was highly significantly lower compared to the insufficient and normal/insufficient groups separately. As for the non-Saudi subjects, the mean neutrophil counts were highly significantly lower for the deficient group compared to the insufficient and normal/insufficient groups separately. All other cell counts and comparisons did not show significant differences.

Vitamin D is known to affect cellular differentiation and growth in the bone marrow and cell development [69 71]. Therefore, the lower neutrophil counts for the lower vitamin D levels found in the current study for both Saudi and non-Saudi subjects comply with the above finding [69,71] of enhanced cellular differentiation and development with higher vitamin D levels. On the other hand, the higher RBC counts found in the present study for the lower vitamin D levels does not comply with these findings [69,71] since the RBC counts for Saudi, but not non-Saudi, subjects were significantly higher for the lower vitamin D levels. Nor do the current findings agree with the previous study [68] that did not find a relationship between the vitamin D levels and RBC counts. On the other hand, these findings agree with the previous finding Doudin, Becker, Rothenberger, and Meyer [72] of lower RBC counts for higher vitamin D levels. In addition, the RBC counts were higher for non-Saudis compared to Saudis for the vitamin D groups, which may mean that there is a difference due to ethnic differences. Hemoglobin concentrations were not different for the different vitamin D groups nor between Saudi and non-Saudi subjects. A previous study Doudin et al. [72] found that higher vitamin D levels led to lower hemoglobin concentrations. Other previous studies found associations between vitamin D levels and hemoglobin although these studies were on patients with renal disease and chronic kidney disease [73], on hemodialysis [74], and requiring cardiac surgery [75] or other diseases or conditions, thus it is not possible to compare these findings with the current ones. A previous study found differences in the presence or lack of the relationship between vitamin D and hemoglobin in black and white children Atkinson et al. [76]. Platelet counts did not show any significant differences between the vitamin D groups nor between Saudi and non-Saudi subjects. This is contradictory with the previous studies [77,78] that found a negative correlation between platelet counts and vitamin D levels.

The counts for WBC and the types of white blood cells were all not significantly different between the vitamin D groups except for the neutrophil counts in Saudi and non-Saudi subjects while not between Saudis and non-Saudis for the counts for each vitamin D level group. These results agree with the findings of other studies [68-80] of no significant effects of vitamin D levels on the total and differential counts of WBC except for the neutrophil counts which disagree. Other studies found an association between the vitamin D levels and WBC counts [81]. In contradiction with the current findings, other studies [82] found a negative association between vitamin D levels and neutrophil and monocyte counts, and higher eosinophil counts with lower vitamin D levels [83, 84]. A study found that the counts of basophils and neutrophils are inversely correlated with the vitamin D levels [84] in contradiction to the present findings. Most other studies found no significant associations between vitamin D levels and eosinophils [85-91] which is in agreement with the present findings. In summary, the lifestyle questionnaire displayed several differences between the Saudi and non-Saudi subjects. The major differences were that Saudi vitamin A deficient subjects was the group that had the greatest number of subjects that got sun exposure daily and ate more immunity enhancing foods and foods containing vitamin D. In addition, RBC counts for the Saudi subjects were significantly higher for lower vitamin D levels, while for non-Saudi subjects there was no dependence on vitamin D levels. Saudi subjects had lower RBC counts compared with non-Saudi subjects for the normal and insufficient vitamin D groups. Neutrophil counts for both Saudi and non-Saudi subjects were lower for lower vitamin D levels. On the other hand, neutrophil counts were not different between Saudi and non-Saudi subjects. In conclusion, low levels of vitamin D (insufficient and deficient) did not cause major changes in the hematology of both groups of subjects and the existent changes were alike in both groups. Thus, it may be concluded that the subject's immune system was not affected considerably by the sub-normal levels of vitamin D and that both Saudi and non-Saudi subjects were affected in the same way. This may be partially due to the fact that the subjects were healthy and middle-aged not old and with diseases. We recommend that further studies be done to determine the concentrations of different inflammatory markers using a larger number of subjects and to determine the vitamin D levels in samples other than blood.

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Conflicts of Interest

There were no conflicts of interest.

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