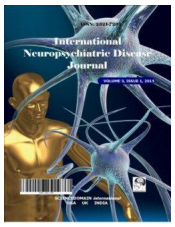

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Hypovitaminosis D Showed Gender and Seasonal Variations in Saudi Patients with Multiple Sclerosis

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Authors' contributions

This work was carried out in collaboration between all authors. Authors OAAS, MES and AMA designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors ZMA, AA and WHK managed the analyses of the study. Authors AMA, WHK and AA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: Multiple sclerosis (MS) is a chronic, inflammatory, autoimmune, demyelinating disease affecting the central nervous system. Vitamin D is an immunomodulating factor with potentiality to prevent and treat MS.
Objectives: To estimate serum levels of 25-hydroxyvitamin D (25-OH-D) in MS patients and to evaluate its seasonal variation and gender dependence.
Patients and Methods: All patients underwent evaluation by using the Neurological Rating Scale (NRS), Expanded Disability Status Scale (EDSS). All patients had radiological work-up and gave two blood samples, one at winter and the second at summer for estimation of serum 25-OH-D levels.
Results: The study included 82 patients. Wintertime serum 25-OH-D levels were significantly lower compared to summertime levels in both sexes with negative significant correlation with age and

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home stay duration, but showed positive significant correlation with being employed. The duration of home stay was a sensitive predictor for low wintertime serum 25-OH-D levels in both males and females, while age and being unemployed in males and being Saudi and unemployed in females were specific predictors for low wintertime serum 25-OH-D levels. The female, age and long duration of home stay were significant predictors for low wintertime serum 25-OH-D levels. Wintertime serum 25-OH-D showed positive significant correlation with both NRS scorings in both males and females, while showed negative significant correlation with EDSS scorings in both sexes. **Conclusion:** Serum vitamin D levels showed seasonal and gender dependence. Also, it could predict MS progression manifested by activity and disability scoring.

Keywords: Multiple sclerosis; vitamin D; seasonal variation; gender-dependence.

1. INTRODUCTION

Multiple sclerosis (MS) is a chronic, inflammatory, and demyelinating disorder that affects the CNS. MS is a common cause of neurological disability in young adults aged 20 to 40. However, 1.1% to 12% of MS patients experience their first symptoms after the age of 50, and this group is defined as late-onset MS. Currently there are more than 1.3 million individuals all over the world suffering from this disease [1].

Studies conducted in the Middle East in recent years indicate increasing spread of MS in these areas where the incidence rate of MS increased significantly from 0.68 in 1989 in 2.93 in 2008 among 100,000 individuals. Several hypotheses attempted to determine underlying causes for increased prevalence of MS in Arabian countries. Individual variables such as gender, family records, consanguinity, life style with special regard to sun exposure, induced change of climate through the dependence on air-conditioning systems were evaluated. In fact, vitamin D deficiency has been recently noted in the Gulf region despite the area's sunny climate [2-5].

The percentage of adults achieving vitamin D sufficiency as defined by 25-hydroxyvitamin (25-OH-D) level of at least 30 ng/ml has declined from about 60% in 1988–1994 to approximately 30% in 2001–2004 in whites and from about 10% to approximately 5% in African Americans during this same time as determined by the Centers for Disease Control and Prevention [6].

Epidemiological data also indicate a low vitamin D status is involved in many autoimmune disorders such as inflammatory bowel disease, rheumatoid arthritis, and multiple sclerosis [7-9].

The current prospective comparative study aimed to estimate serum levels of 25-OH-D in MS patients and to evaluate its seasonal variation, gender dependence and relation to demographic, clinical and radiological data.

2. PATIENTS AND METHODS

The current study was conducted at Departments of Neurology, Diagnostic Radiology and Rheumatology at Saudi German Hospital, KSA since Jan 2012 till Jan 2015. The study protocol was approved by the Local Ethical Committee at the hospital. All enrolled patients had signed written fully informed consent to participate in the study. MS patients included according to McDonald diagnostic criteria 2010.

Collected demographic information, and disease-related data were recorded and analyzed. Clinical evaluation includes Scripps Neurological Rating Scale (NRS), and Kurtzke Expanded Disability Status Scale (EDSS) [10,11]. All patients had radiological work-up including MRI Brain, spine and MRI spectroscopy. All patients gave two blood samples, one at winter and the second at summer for evaluation of the seasonal variation of serum 25-OH-D levels. Samples were adjusted mostly at Jan and July when the change in temperature was the most. Blood samples obtained under aseptic conditions, allowed to clot and serum was collected Eppendorf tubes and stored at -80ºC till be assayed for serum 25-OH-D levels using BioSource 25OH-Vit. D3-Ria-CT Kit (BioSource Europe S.A., Rue de L'Industrie, 8, B-1400 Nivelles, Belgium) [12]. Patients were evaluated for NRS and EDSS on two occasions mostly at time of blood sampling. Patients were categorized into two groups according to gender for evaluation of the impact of gender-related demographic data on functional and disability status and its relation to estimated serum 25-OH-D levels.

2.1 Statistical Analysis

Obtained data were presented as mean±SD, ranges, numbers and ratios. Results were analyzed using Wilcoxon multivariate analysis; ranked test for unrelated data (Z-test) for inter-group comparisons and paired t-test for intragroup comparisons and Chi-square test (X2 test) for non-parametric analysis of numbers and ratios using Friedman test. ROC curve analysis defined duration of home stay as a sensitive predictor for low wintertime serum 25-OH-D levels. Statistical analysis was conducted using the SPSS (Version 15, 2006; SPSS Inc., Chicago, IL, USA) for Windows statistical package. P value <0.05 was considered statistically significant.

3. RESULTS

The study included 82 patients; 26 males and 56 females with male: female ratio of 1: 2.2 and had mean age of 37.4±8.9, range: 24-62 years. Patients' characteristics as regards age, ethnicity, marital status, literacy, employment and duration of home stay showed significant difference between males and female patients, while marital status showed non-significant difference between males and females (Table 1).

Disease-related data showed non-significant (p>0.05) difference between males and females, apart from the significantly higher frequency of female patients among strata of longer disease duration and dependence on steroid therapy (Table 2).

Serum 25-OH-D levels estimated at wintertime were significantly (Z=7.843, p=0.0005) lower compared to that estimated at summertime, irrespective of gender (Fig. 1). Serum 25-OH-D levels estimated at summertime were significantly higher compared to that estimated at wintertime in both males (t=13.39; p=0.0009) and females (t=20.190; p=0.0004). Males showed significantly higher serum 25-OH-D levels compared to females both at wintertime (Z=2.162, p=0.031) and summertime (Z=2.302; p=0.021). However, the percentage of increase of serum 25-OH-D levels was significantly higher in females compared to males (Fig. 2). Moreover, the frequency of patients had serum 25-OH-D levels <20 ng/ml was significantly higher in winter-sample compared to summer-sample in both sexes with significantly higher frequency of patients had serum 25-OH-D levels <20 ng/ml among females during summer with non-significant difference during winter (Table 3, Fig. 3).

Table 1. Demographic data of studied patients categorized according to gender

			Males (n=26)	Females (n=56)	Statistical significance
Age (years)	Strata	<30	8 (30.8%)	11 (30.4%)	X^2=2.517, p>0.05
		>30-	7 (26.9%)	23 (41.1%)	
		>40-	10 (38.5%)	12 (21.4%)	
		>50	1 (3.8%)	4 (7.1%)	
	Total		38.3±8.7	34±8.8	Z=2.351, p=0.019
Ethnicity	Saudi		14 (53.8%)	39 (69.6%)	X^2=3.591, p=0.039
	Immigrant		12 (46.2%)	17 (30.4%)	
Marital status	Married		17 (65.4%)	42 (75%)	X^2=0.102, p>0.05
	Single		5 (19.2%)	8 (7.1%)	
	Widowed		2 (7.7%)	4 (7.3%)	
	Divorced		2 (7.7%)	2 (3.6%)	
Literacy	Illiterate		4 (15.4%)	11 (19.6%)	X^2=3.261, p=0.041
	Elementary		6 (23.1%)	23 (41.1%)	
	High school		11 (42.3%)	15 (26.8%)	
	University		5 (19.2%)	7 (12.5%)	
Employment	Employed		19 (73.1%)	39 (55.4%)	X^2=3.365, p=0.04
	Unemployed		7 (26.9%)	25 (44.6%)	
Duration of home stay (hours/day)	<12		15 (57.7%)	16 (28.5%)	X^2=5.494, p=0.001
	12-18		9 (34.6%)	17 (30.4%)	
	18-24		2 (7.7%)	23 (41.1%)	

Data are presented as numbers & mean±SD; percentages & ranges are in parenthesis; p<0.05: significant difference

Table 2. Disease-related data of studied patients categorized according to gender

			Males (n=26)	Females (n=56)	Statistical significance
Duration of disease (years)	Strata	1-3	14 (53.8%)	18 (32.1%)	X^2=5.78, p=0.017
		>3-5	9 (34.6%)	29 (51.8%)	
		>5	3 (11.6%)	9 (16.1%)	
	Total		3.9±1.4	3.3±1.2	Z=1.985, p>0.05
1st symptoms & signs	Sensory		12 (46.2%)	19 (33.9%)	X^2=1.576, p>0.05
	Kinesthetic		10 (38.5%)	24 (42.9%)	
	Visual		4 (15.3%)	13 (23.2%)	
Positive family history	Yes		8 (30.8%)	22 (39.3%)	X^2=1.812, p>0.05
	No		18 (69.2%)	34 (60.7%)	
Other diseases	Yes		5 (19.2%)	39 (28.6%)	X^2=1.387, p>0.05
	No		21 (80.8%)	40 (71.4%)	
Lines of treatment	Steroid therapy	Yes	6 (23.1%)	22 (39.3%)	X^2=3.904, p=0.035
		No	20 (76.9%)	34 (60.7%)	
	Rehabilitation	Yes	9 (34.6%)	29 (51.8%)	X^2=3.59, p=0.039
		No	17 (65.4%)	27 (48.2%)	
	Alternative remedies	Yes	16 (61.5%)	27 (48.2%)	X^2=3.766, =0.037
		No	10 (38.5%)	29 (51.8%)	
Disease progression type	Relapsing-remitting		18 (69.2%)	35 (62.5%)	X^2=0.586, p>0.05
	1ry progressive		3 (11.6%)	8 (14.3%)	
	2ry progressive		5 (19.2%)	13 (23.2%)	

Data are presented as numbers & mean±SD; percentages & ranges are in parenthesis; p<0.05: significant difference

Table 3. Serum 25-OH-D estimated in studied patients categorized according to gender estimated at winter and summer

Season	Males (n=26)	Females (n=56)	Statistical analysis
Winter (ng/ml)	20.2±6.7	18.7±7.8	Z=2.162, p=0.031
Summer (ng/ml)	25.9±7.6	24.1±8.7	Z=2.302; p=0.021
Statistical analysis	t=13.39; p=0.0009	t=20.190; p=0.0004	

Data are presented as mean±SD

Serum 25-OH-D levels estimated at wintertime showed negative significant correlation with age and home stay duration, but showed positive significant correlation with being employed, irrespective of gender. In males, serum 25-OH-D levels estimated at wintertime showed negative significant correlation with age, being Saudi and home stay duration, but showed positive significant correlation with being employed, while in females, serum 25-OH-D levels estimated at wintertime showed negative significant correlation with age and home stay duration (Table 4).

ROC curve analysis defined duration of home stay as a sensitive predictor for low wintertime

Table 4. Correlation between wintertime serum 25-OH-D estimated in studied patients categorized according to gender and patients' demographic data

	Total patients (n=82)		Male patients (n=26)		Female patients (n=56)	
	"r"	p	"r"	p	"r"	p
Male gender	0.096	>0.05				
Age (years)	-0.599	0.001	-0.814	0.0004	-0.543	0.0008
Married	-0.084	>0.05	-0.035	>0.05	-0.094	>0.05
Saudi	-0.161	>0.05	-0.524	0.006	0.125	>0.05
Illiterate	0.045	>0.05	0.217	>0.05	-0.051	>0.05
Employed	0.248	0.024	0.555	0.003	0.124	>0.05
Home stay duration (hr)	-0.393	0.006	-0.406	0.040	-0.378	0.004

serum 25-OH-D levels in both males and females, while showed that age and being unemployed in males and being Saudi and unemployed in females as specific predictors for low wintertime serum 25-OH-D levels (Table 5).

Regression analysis defined being female, age and long duration of home stay as significant predictors for low wintertime serum 25-OH-D levels in one model and age as significant predictors for low wintertime serum 25-OH-D levels (Table 6).

Wintertime serum 25-OH-D showed positive significant correlation with both NRS scorings in both males and females, while showed negative significant correlation with EDSS scorings in both sexes (Table 7).

4. DISCUSSION

East countries are a proper model of hot-dry climate that previously known to have low frequency of MS. Bohlega and his colleagues documented that the Arabian Gulf Region is located in a low-risk zone for MS; however, recent studies suggest a moderate-to-high prevalence [13]. Al-Tahan and his colleagues found that there is a substantial burden of MS [14]. Also, Alroughani and his colleagues found the incidence of MS in Kuwait had increased 3.22 and 2.54 times in women and men respectively [15].

Table 5. Area under curve of ROC curve analysis of patients' demographic data as predictors for wintertime serum 25-OH-D ≤24 ng/ml

	Total	Males	Females
Gender	V1 0.340		
Age (years)	V4 0.642	V9 0.760	V19 0.815
Married	V7 0.321	V10 0.220	V17 0.398
Saudi	V2 0.556	V13 0.640	V20 0.472
Illiterate	V5 0.256	V11 0.160	V18 0.370
Employed	V3 0.679	V12 0.700	V21 0.426
Home stay duration (hr)	V6 0.191	V14 0.001	V16 0.069

Table 6. Regression analysis of patients' demographic data as predictors for wintertime serum 25-OH-D ≤24 ng/ml

	Model 1			Model 2		
	β	t	p	β	t	p
Female gender	0.180	2.036	0.045	0.093	1.029	>0.05
Age (years)	-0.543	6.323	0.0005	-0.599	6.694	0.0004
Married	-0.002	-0.018	>0.05	0.030	0.335	>0.05
Saudi	-0.125	-1.396	>0.05	-0.085	-1.002	>0.05
Illiterate	0.004	0.047	>0.05	-0.008	-0.093	>0.05
Employed	0.162	1.185	>0.05	0.064	0.697	>0.05
Home stay duration (hr)	-0.288	-3.349	0.001	0.183	2.109	>0.05

Table 7. Correlation between wintertime serum 25-OH-D estimated in studied patients categorized according to gender and patients' neurological rating scale and expanded disability status scale

	Total patients (n=82)		Male patients (n=26)		Female patients (n=56)	
	"r"	p	"r"	p	"r"	p
Neurological rating scale	0.432	0.0009	0.486	0.012	0.336	0.011
Expanded disability status scale	-0.372	0.001	-0.432	0.028	-0.399	0.002

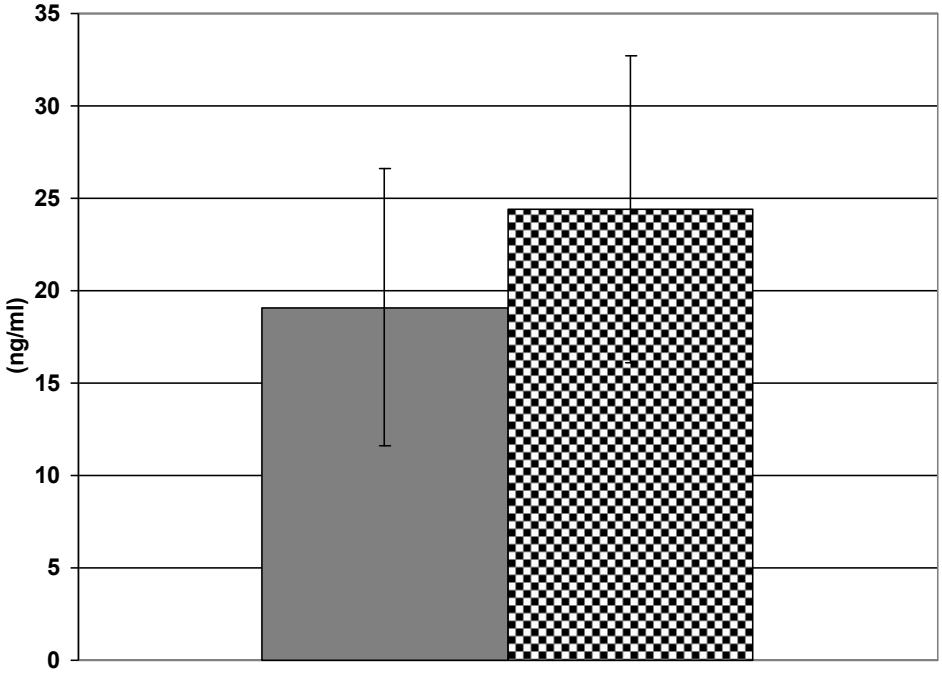

Fig. (1): Mean (±SD) serum levels of 25-OH-D estimated at winter and summer for studied patients

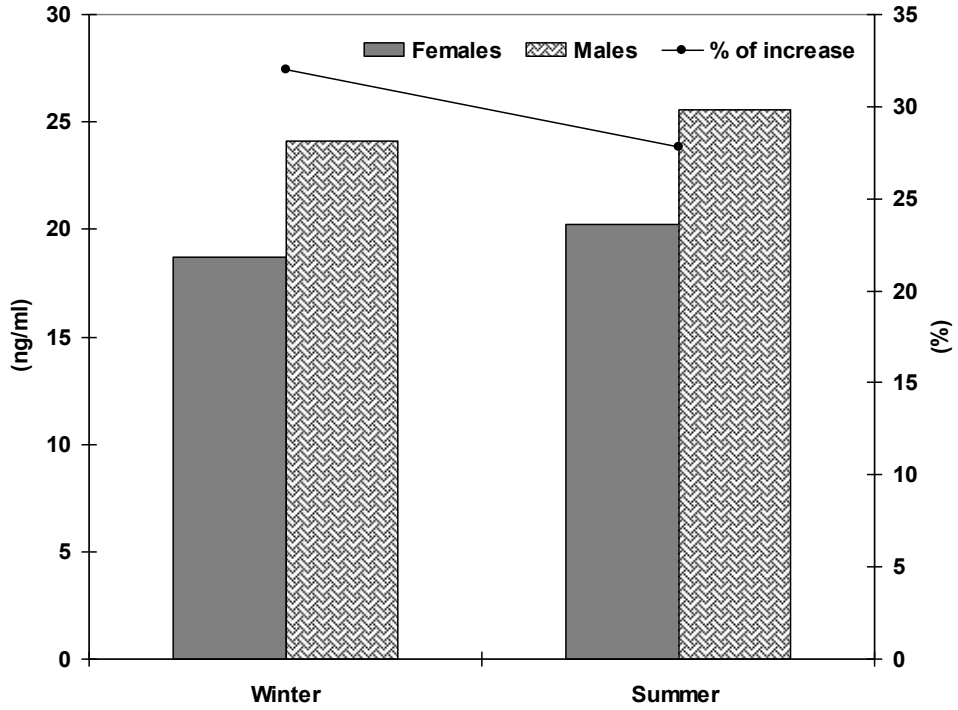

Fig. (2): Mean serum 25-OH-D levels estimated in studied patients categorized according to gender at winter and summer with the percentage of increase in levels

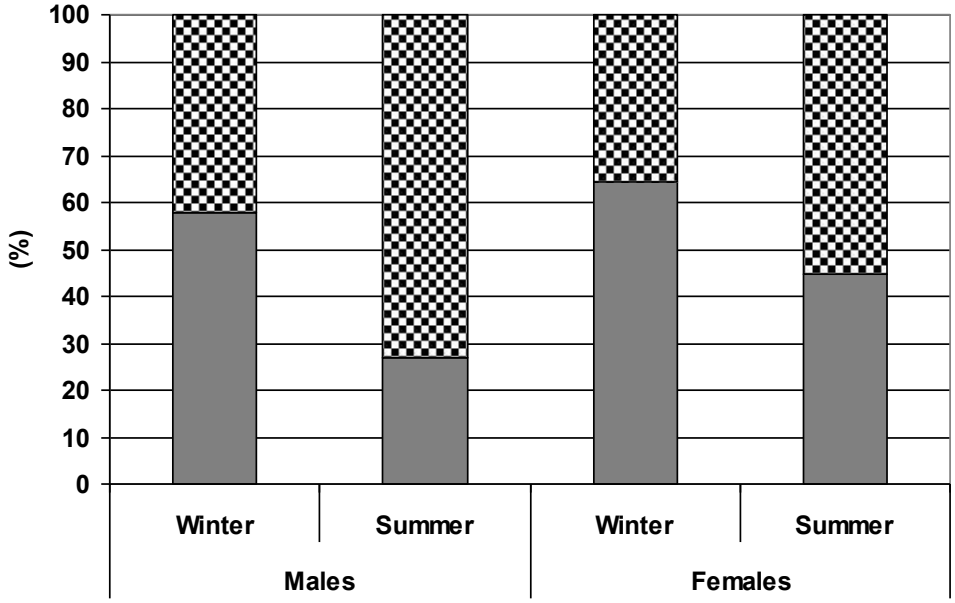

Fig. (3): Patients' distribution according to serum 25-OH-D <20 ng/ml estimated at both winter and summer and categorized according to gender

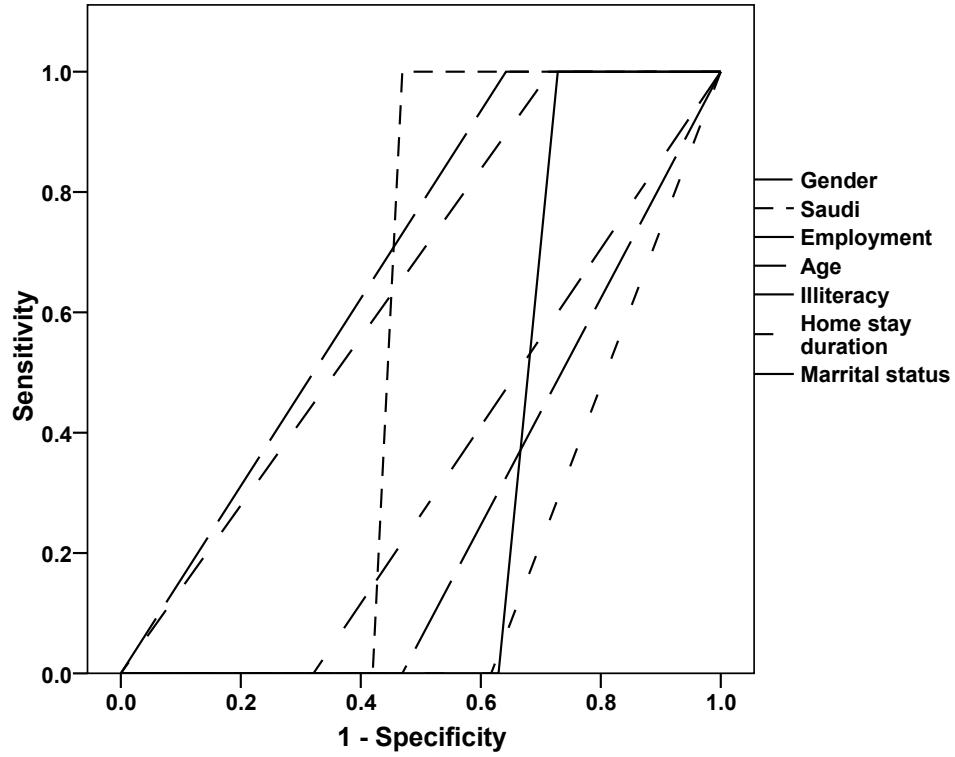

Fig. 4. ROC curve analysis of patients' demographic data as predictors for wintertime serum 25-OH-D ≤24 ng/ml in studied patients

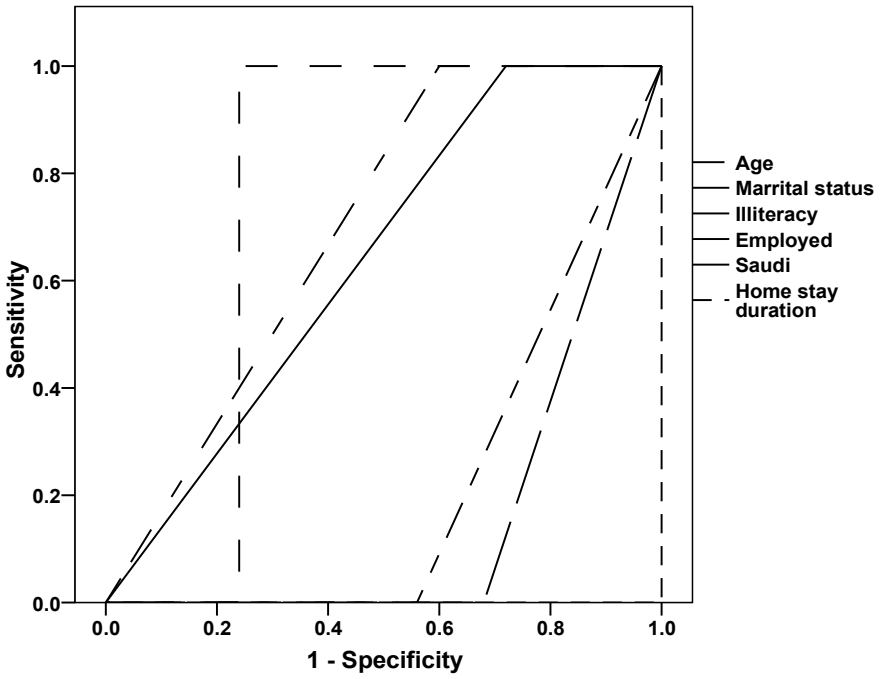

Fig. 5. ROC curve analysis of patients' demographic data as predictors for wintertime serum 25-OH-D ≤24 ng/ml in studied male patients

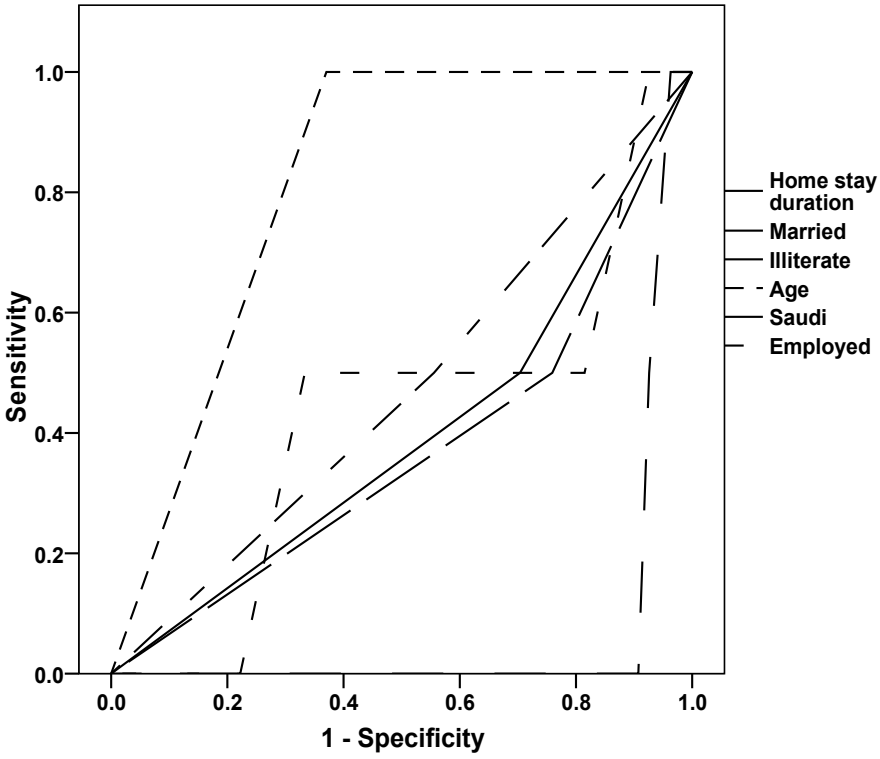

Fig. 6. ROC curve analysis of patients' demographic data as predictors for wintertime serum 25-OH-D ≤24 ng/ml in studied female patients

The male to female ratio was 1:2.2, such ratio approached to that reported in cold-climate countries where Zsiros and his colleagues reported a male: female ratio of 1:2.9 [16]. Yaouanq and his colleagues documented that Brittany is confirmed as a high-risk region for MS [17].

Mean age of studied patients was 37.4±8.9, but females were significantly younger than males; such mean age goes in hand with Alroughani and his colleagues who showed that mean age of studied MS patients in Kuwait was 35.4±10.99 years [18]. Such mean age of patients in KSA and Kuwait is higher than that reported in cold climate countries as the mean age of onset of MS patients in La Rioja, Spain, was 20-29 years [18]. Heydarpour and his colleagues compared the age in a meta-analysis of 52 studies and the mean age at disease onset ranged from 25.2 years in Kuwait to 32.5 years in Northeastern Iran, with an overall estimate of 28.54 years [19].

Serum levels of 25-OH-D in studied patients showed seasonal and gender difference in both winter and summer samples with significantly lower frequency of patients had serum levels <20 ng/ml in males vs. females in summer. Moreover, serum 25-OH-D levels showed negative significant correlation with both of age and duration of home stay, while showed positive significant correlation with employment. In line with these findings, Salzer and his colleagues supported the presence of an association between high 25-OH-D levels during the years preceding disease onset and a decreased risk of MS [20]. Holmøy and his colleagues recommended that MS patients are supplemented with 800 IU of vitamin D at least from autumn to spring [21].

In line with these findings, Steffensen and his colleagues found half of their study MS patients had sufficient winter vitamin D levels at baseline (≥20 ng/ml) and vitamin D status was predicted by sun exposure during the last 3 months and by ingested vitamin D from diet and supplements [22]. Martinelli and his colleagues found that 52% of patients with clinically isolated syndromes had 25-OH-D level <20 ng/ml and during follow-up, 55 patients developed clinically definite MS (CDMS) and patients had very low and low 25-OH-D levels were particularly at risk of CDMS with hazard risk ratio of 2.12 and 1.61 and concluded that low serum vitamin D is associated with increased MS risk in patients with clinically isolated syndrome [23].

Also, Ascherio found that in individuals presenting with a first demyelinating episode, higher levels of vitamin D, sun exposure or actinic damage were found to be associated with reduced MS risk, the risk of MS was found to be 30% lower among women in the highest quintile of vitamin D intake from diet and supplements compared with those in the lowest quintile and during an average of 5 years' follow-up, MS risk among healthy young adults with serum levels of 25-OH-D vitamin D >40 ng/ml was about 60% lower than in individuals of the same age and sex with serum 25(OH) vitamin D levels <40 ng/ml [24]. Knippenberg and his colleagues found that higher levels of sun exposure, rather than 25-OH-D levels, were associated with less depressive symptoms and levels of fatigue in MS [25].

Statistical analyses defined age and duration of home stay as predictors for low serum 25-OH-D levels at cutoff point of 24 ng/ml and serum 25-OH-D levels showed negative significant correlation with disease activity and disability scorings especially in wintertime. In line with this finding, Ascherio and his colleagues reported that among patients with MS mainly treated with interferon β-1b, higher 25-OH-D levels predicted reduced MS activity with a slower rate of progression; a 20-ng/ml increment in serum 25-OH-D levels within the first 12 months predicted a 57% lower rate of new active lesions and relapse rate with 25% lower yearly increase in T2 lesion volume and 0.41% lower yearly loss in brain volume, and 25-OH-D levels, values ≥20 ng/ml at up to 12 months predicted lower disability during the subsequent 4 years [26].

Interestingly, there is a relationship between diet, gut microbiota, and vitamins D and A in multiple sclerosis is low-grade inflammation, which is involved in all chronic inflammatory diseases. Food components have either proinflammatory or anti-inflammatory effects and influence both the human metabolism (the "metabolome") and the composition of gut microbiota. Hypercaloric, high-animal-fat Western diets favor anabolism and change gut microbiota composition towards dysbiosis. Subsequent intestinal inflammation leads to leakage of the gut barrier, disruption of the blood-brain barrier, and neuroinflammation. Conversely, a vegetarian diet, rich in fiber, is coherent with gut eubiosis and a healthy condition. Vitamin D levels, mainly insufficient in a persistent low-grade inflammatory status, can be restored to optimal values only by administration of high amounts of cholecalciferol.

At its optimal values (>30 ng/ml), vitamin D requires vitamin A for the binding to the vitamin D receptor and exert its anti-inflammatory action. Both vitamins must be supplied to the subjects lacking vitamin D. Nutrients, including the nondigestible dietary fibers, have a leading role in tackling the low-grade inflammation associated with chronic inflammatory diseases. Their action is mediated by gut microbiota and any microbial change induced by diet modifies host-microbe interactions in a consequent way, to improve the disease or worsen it [27].

5. CONCLUSION

Low serum levels of vitamin D may be the underlying etiological cause for development of MS in Arabian countries. Serum vitamin D levels with seasonal and gender dependence is mostly related to traditional dress pattern of females preventing sun exposure. It could predict MS progression manifested by activity and disability scoring.

CONSENT

As per international standard or university standard, patient's written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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