ORIGINAL ARTICLE

Prevalence of Subclinical Carotid Atherosclerosis and Vitamin D Deficiency in Egyptian Ankylosing Spondylitis Patients

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ABSTRACT

Objectives: This study aims to investigate the relationship between subclinical carotid atherosclerosis and vitamin D deficiency in Egyptian ankylosing spondylitis (AS) patients and their impact on disease activity.

Patients and methods: This cross-sectional study included 40 AS patients (36 males, 4 females; mean age 45.9±8.4 years; range 33 to 55 years) diagnosed according to the 1984 modified New York criteria with equal number of healthy controls (26 males, 14 females; mean age 48.4±7.8 years; range 31 to 55 years). Patients' histories were taken and clinical examinations were performed. Disease activity was assessed with Bath AS metrology index (BASMI), Bath AS disease activity index (BASDAI), and Bath AS functional index (BASFI) scores. Laboratory evaluation included lipid profile and 25-hydroxyvitamin D [25(OH)D] was determined by enzyme-linked immunosorbent assay. Bilateral carotid intima-media thickness (CIMT) was measured by a high-resolution ultrasound with linear 7-12 MHz transducer. Average of CIMT of right and left common carotid arteries was used.

Results: Statistically significant differences were found between patients and controls in terms of CIMT ($p<0.001$), 25(OH)D3 ($p<0.001$) and triglycerides ($p=0.02$). A significant positive correlation was present between CIMT and disease duration ($r=0.74$), disease activity scores [BASFI ($r=0.60$), BASMI ($r=0.49$), BASDAI ($r=0.65$)] and lipid profile except for high-density lipoprotein (HDL) that had a negative correlation ($r=-0.52$). A significant negative correlation was present between 25(OH)D3 levels and CIMT ($r=-0.38$) and lipid profile except for HDL having a positive correlation ($r=0.40$).

Conclusion: Prevalence of subclinical carotid atherosclerosis in AS patients compared to the healthy population was associated with high disease activity and functional limitations. In AS patients, 25(OH)D3 deficiency is a risk factor for accelerated atherosclerosis.

Keywords: 25-hydroxyvitamin D, carotid intima, high-density lipoproteins, thickness, triglycerides.

Ankylosing spondylitis (AS) is a systemic inflammatory disorder of unknown etiology that mainly involves the axial skeleton causing spine, sacroiliac joints arthritis, and peripheral joints arthritis. Its peak age of onset is between 20-30 years affecting young males with involvement of extra-articular structures such as eyes, kidneys, heart, lung, vessels, and nerves.[1,2]

The initial symptom is usually dull pain in the lower lumbar and gluteal region insidious in onset, associated with morning stiffness of low back up to few hours with improvement with activity and worsening following inactivity.[1]

Aortitis and aortic regurgitation are cardiovascular complications associated with AS. AS is associated with up to 50% mortality rates and cardiovascular diseases are the main causes of these high mortality rates.[2,3]

Sustained chronic systemic inflammation predispose to atherosclerosis as it is accompanied

by elevated levels of serum C-reactive protein (CRP), which has proatherogenic effects. Also, hypertension, sex and smoking are predisposing factors with chronic inflammation in atherosclerosis.[3]

Moreover, AS has been associated with a prevalence of metabolic syndrome including dyslipidemia and high ratio of low-density lipoprotein (LDL) cholesterol to high-density lipoprotein (HDL) cholesterol.[4,5]

High-resolution ultrasonography is effective in detecting subclinical atherosclerosis by carotid intima-media thickness (CIMT) measurement. High CIMT correlates with atherosclerotic loading.[6,7]

On the other hand, 25-hydroxyvitamin D3 [25(OH)D3] has numerous physiological functions and a significant immune-modulatory effect. Its deficiency is a very common health problem that affects about 50% of the general population.[3,8] Several studies have indicated that deficiency of vitamin D is a marker of cardiovascular disease (CVD) risk, as low serum levels of 25(OH)D3 were associated with CVD risk factors or CIMT.[9] Vitamin D may indirectly predispose to atherosclerosis as it is related to HDL levels.[8,10]

In this study, we aimed to investigate the relationship between subclinical carotid atherosclerosis and vitamin D deficiency in Egyptian AS patients and their impact on disease activity.

PATIENTS AND METHODS

This cross-sectional study included 40 patients (36 males, 4 females; mean age 45.9±8.4 years; range 33 to 55 years) diagnosed as AS according to the 1984 modified New York criteria of AS[11] with an equal number of healthy controls (26 males, 14 females; mean age 48.4±7.8 years; range 31 to 55 years) matched for age and sex and recruited from Menoufia University Outpatient Clinic of Physical Medicine, Rheumatology and Rehabilitation Department. between March 2018 and January 2019. Exclusion criteria were patients with transient ischemic attack, chronic renal disease (serum creatinine >1.2 mg/dL), percutaneous luminal coronary angioplasty, surgery for ischemic heart disease, cerebrovascular disease (confirmed by computed tomography scan

or magnetic resonance imaging of the brain), and patients on vitamin D supplementation. The study protocol was approved by the Menoufia University Hospital Ethics Committee. A written informed consent was obtained from each participant. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Patients' demographic data were collected as well as their histories. Clinical examinations included general examination and musculoskeletal examinations of both axial and peripheral joints. AS disease activity was assessed by the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI),[12] Bath Ankylosing Spondylitis Metrology Index (BASMI)[13] and Bath Ankylosing Spondylitis Functional Index (BASFI) scores.[14] Pain was recorded on a 10-cm visual analog scale (VAS).[15]

After fasting for at least eight hours, venous blood sample was obtained from all subjects for the measurement of serum creatinine, erythrocyte sedimentation rate and CRP, and lipid profile [total cholesterol, HDL, LDL and triglycerides (TG)]. Level of 25(OH)D was measured by enzyme-linked immunosorbent assay using a homogenous enzyme-coupled vitamin D binding protein to measure true total level of 25(OH)D3. This protein recognizes vitamin D2 and D3 equally and also recognizes the true total level of 25(OH)D3. Serum levels of 25(OH)D3 less than 15 ng/mL are considered as vitamin D deficiency.[16]

Bilateral assessment of CIMT was performed using high-resolution ultrasonography (Philips-HD11 XE with multi-frequency linear 7-12 MHz transducer, Koninklijke Philips Electronics N.V. USA) after 15 minutes rest and the participants were examined in supine position with neck extended and chin turned contralateral to the side being examined. The scanning technique and the setting of the machine were the same for all participants and all examinations were performed by the same radiologist. Average of CIMT of right and left common carotid arteries was used. CIMT ranging from 0.59-0.95 mm was considered abnormal while 1.0 mm or more was considered high risk.[17]

Statistical analysis

The data collected were analyzed by the IBM SPSS version 20.0 software (IBM Corp., Armonk,

NY, USA). Quantitative data were expressed as mean, standard deviation and range and analyzed by independent samples t-test. Pearson correlation coefficient test was assessed between different variables. Chi-squared test was used to study the association between two qualitative variables. *P* value ≤0.05 was considered significant while *p* value ≤0.001 was considered statistically highly significant.[18]

RESULTS

The mean disease duration for the patient group was 10.4±5.0 years (Table 1). There were statistically significant differences in CIMT, 25(OH)D3 levels and lipid profile regarding TG (p=0.02) between patients and controls. Patients had higher CIMT than the controls (0.6±0.1 vs. 0.6±0.0) and lower levels of 25(OH)D3 (p<0.001) with lower levels of HDL and higher levels of cholesterol and TG. LDL levels were higher in controls compared to patients (Table 1).

This study revealed a significant negative correlation between CIMT and 25(OH)D3 levels (r=-0.38) and a significant positive correlation between CIMT and disease duration in AS patients (r=0.74) (Table 2).

In AS patients, CIMT measurements had a positive correlation with disease activity assessed by BASMI, BASFI and BASDAI scores (r=0.49, p=0.002; r=0.60, p<0.001; r=0.65, p=0.001, respectively) and also a positive correlation with lipid profile regarding cholesterol, LDL and TG (r=0.25, p=0.11; r=0.49, p=0.002; r=0.57, p<0.001, respectively) while having a negative correlation with HDL (r=-0.52, p<0.001) (Table 2).

In AS patients, there was a statistically significant negative correlation between serum levels of 25(OH)D3 and disease activity scores regarding BASDAI, BASMI and BASFI (r=-0.69, p<0.001; r=-0.41, p=0.008; r=-0.71, p<0.001, respectively). There was a negative correlation with lipid profile regarding cholesterol, LDL and TG (r=-0.45, p=0.003; r=-0.77, p<0.001;

Table 1. Demographic and laboratory data of studied groups

	Studied groups							
	Patients (n=40)			Controls (n=40)				
	n	%	Mean±SD	n	%	Mean±SD	Test of significance	p
Age (year)			45.9±8.4			48.4±7.8	t- test =1.36	0.17
Sex							χ²=7.17	0.007
Male	36	90.0		26	65.0			
Female	4	10.0		14	35.0			
Smoking							χ²=0.81	0.37
Smoker	25	62.5		21	52.5			
Non-smoker	15	37.5		19	47.5			
Disease duration (year)			10.4±5.0			-		-
Total cholesterol level (mg/dL)			157.3±18.7			156.4±11.5	t= 0.27	0.78
Triglycerides (mg/dL)			123.7±31.3			107.3±29.6	t=2.41	0.02
High-density lipoproteins (mg/dL)			42.2±6.1			43.0±6.2	t=0.58	0.56
Low-density lipoproteins (mg/dL)			99.7±11.7			103.3±10.1	t=1.45	0.15
Vitamin D (ng/mL)			16.9±4.0			20.3±3.6	t=3.93	<0.001
Vitamin D (ng/mL)							χ²=5.49	0.01
Deficient (≤20)	31	77.5		21	52.5			
Insufficient (21-29)	9	22.5		19	47.5			
Carotid intima-media thickness (mm)			0.6±0.1			-		-
BASDAI			5.1±1.1			-		-
BASFI			49.7±11.6			-		-
BASMI			4.6±0.8			-		-
Visual analog scale			7.0±1.1			-		-

SD: Standard deviation; BASDAI: Bath ankylosing spondylitis disease activity index; BASFI: Bath ankylosing spondylitis functional index; BASMI: Bath ankylosing spondylitis metrology index; VAS: χ²: Chi-squared test.

Table 2. Correlation between carotid intima-media thickness and laboratory measurements among studied groups

| | Carotid intima-media thickness (mm) | | | |
| | Patients (n=40) | | Controls (n=40) | |
	*r	p	*r	p
Disease duration (year)	0.74**	<0.001	-	-
Total cholesterol level (mg/dL)	0.25	0.11	0.92	<0.001
Triglycerides (mg/dL)	0.57	<0.001	0.94	<0.001
High-density lipoproteins (mg/dL)	-0.52	<0.001	- 0.93	<0.001
Low-density lipoproteins (mg/dL)	0.49	0.002	0.91	<0.001
Vitamin D (ng/mL)	-0.38	0.01		
BASDAI	0.65	<0.001	-	-
BASFI	0.60	<0.001	-	-
BASMI	0.49	0.002	-	-
Visual analog scale	0.64	<0.001	-	-

* Pearson correlation coefficient; ** Spearman correlation coefficient; BASDAI: Bath ankylosing spondylitis disease activity index; BASFI: Bath ankylosing spondylitis functional index; BASMI: Bath ankylosing spondylitis metrology index.

r=-0.59, p<0.001, respectively) while there was a significant positive correlation with HDL levels (r=0.40, p=0.01) (Table 3).

In AS patients, statistically significant differences were found between vitamin D deficient and vitamin D insufficient patients regarding disease duration (p<0.001) and lipid profile (cholesterol, TG and LDL) (Table 4).

In AS patients, statistically significant differences were found between vitamin D deficient and vitamin D insufficient patients regarding CIMT (p<0.001), disease activity regarding BASDAI, BASFI and BASMI (p<0.001, p<0.001 and p=0.02, respectively) and VAS (p<0.001) (Table 4).

DISCUSSION

Ankylosing spondylitis like other autoimmune diseases is associated with increased risk of atherosclerosis due to the chronic inflammatory conditions existing with this disorder. Male

Table 3. Correlation between vitamin D and laboratory measurements among studied groups

| | Vitamin D (ng/mL) | | | |
| | Patients (n=40) | | Controls (n=40) | |
	*r	p	r	p
Disease duration (year)	-0.68**	<0.001	-	-
Total cholesterol level (mg/dL)	-0.45	0.003	-0.87	<0.001
Triglycerides	-0.59	<0.001	-0.91	<0.001
High-density lipoproteins	0.40	0.01	0.88	<0.001
Low-density lipoproteins	-0.77	<0.001	-0.89	<0.001
BASDAI	-0.69	<0.001	-	-
BASFI	-0.71	<0.001	-	-
BASMI	-0.41	0.008	-	-
Visual analog scale	-0.62	<0.001	-	-

* Pearson correlation coefficient; ** Spearman correlation coefficient; BASDAI: Bath ankylosing spondylitis disease activity index; BASFI: Bath ankylosing spondylitis functional index; BASMI: Bath ankylosing spondylitis metrology index.

Table 4. Relationship between vitamin D and laboratory parameters among studied ankylosing spondylitis patients (n=40)

| | Vitamin D (ng/mL) | | | |
| | Deficient (n=31) | Insufficient (n=9) | | |
	Mean±SD	Mean±SD	Mann-Whitney test	p
Disease duration (year)	11.9±4.7	5.2±1.0	3.83	<0.001
Total cholesterol level (mg/dL)	160.6±18.1	146.0±16.9	2.12	0.03
Triglycerides	130.3±28.1	100.9±32.6	2.28	0.02
Low-density lipoproteins	103.2±10.6	87.9±6.4	3.58	<0.001
High-density lipoproteins	40.5±5.5	48.1±4.1	3.17	0.002
Carotid intima-media thickness (mm)	0.6±0.1	0.6±0.0	3.51	<0.001
BASDAI	5.5±1.0	4.0±0.6	4.23	<0.001
BASFI	53.2±10.5	37.7±5.1	3.63	<0.001
BASMI	4.7±0.7	4.1±0.6	2.27	0.02
Visual analog scale	7.4±0.8	5.7±0.7	3.97	<0.001

SD: Standard deviation; BASDAI: Bath ankylosing spondylitis disease activity index; BASFI: Bath ankylosing spondylitis functional index; BASMI: Bath ankylosing spondylitis metrology index.

to female ratio of the AS patients was 9:1, which is consistent with the usual pattern of the disease.[19,20] Vitamin D has a significant immunomodulatory effect and many physiologic functions while many studies have proven that vitamin D deficiency is a risk factor for cardiovascular disease. Thus the relationship between AS, 25(OH)D3 deficiency and atherosclerosis is significant to be studied.[1,9]

Egyptian AS populations may differ from other populations having special dietary habits with high fat content and also having higher cardiovascular morbidity and mortality. This enhanced risk of atherosclerosis when combined with the chronic inflammatory state in AS leads to high prevalence of atherosclerosis in Egyptian AS patients.[21]

This study has revealed a statistically significant increase in CIMT (p<0.001) in AS patients except than controls except LDL and HDL were lower in AS patients than controls (42.2±6.1 and 99.7±11.7 vs. 43.0±6.2 and 103.3±10.1, respectively). Decreased 25 (OH)D3 levels were detected in AS patients compared to controls (p<0.001) (Table 1).

In accordance to these results, Berg et al.,[5] Skare et al.[22] and Gupta et al.[23] have reported significantly increased CIMT in AS patients compared with controls. Schieir et al.[4] and Mathieu et al.[2] have also documented dyslipidemia

and high levels of cholesterol with low levels of LDL in AS patients compared to controls (81.0±21.7 vs. 107.4±25.8, respectively). This is due to the chronic inflammation which causes structural modifications in lipid molecules making them more atherogenic.[4]

Contrary to our results, Arida et al.,[24] Geçene et al.[25] and Choe et al.[26] have found non-significant differences in CIMT between AS patients and controls. This can be explained by the younger age groups they have studied as their mean age was 31.8±6.8 years.

Our study has revealed that in AS patients, CIMT measurements were positively correlated with disease activity scores, BASMI, BASFI and BASDAI (r=0.49, r=0.60, r=0.65, respectively) and with lipid profile regarding cholesterol, LDL and TG (r=0.25, r=0.49, r=0.57, respectively) while being negatively correlated with HDL (r=-0.52) (Table 2).

In accordance to our results, Sharma et al.[6] and Valente et al.[27] have reported a significant positive relationship between CIMT and lipid profile (cholesterol, LDL, and TG). They reported that AS patients are at risk of increased CIMT (0.6±0.1) and therefore atherosclerosis. On the other hand, Perrotta et al.[28] have reported non-significant differences regarding CIMT measurements in AS patients.

This study has demonstrated a negative correlation between CIMT and 25(OH)D3 levels (r=-0.38) and a positive correlation between CIMT and disease duration and VAS (r=0.74, r=0.64) in AS patients (Table 2). This was comparable to findings of Perrotta et al.[28] and Briot et al.[29] as they have reported that low 25(OH)D3 levels in AS are associated with increased CIMT. Perrotta et al.[28] have also reported that AS patients with high disease activity (mean BASDAI=6.01) and long disease duration (range, 1-36 years) are at risk of increased CIMT and consequently atherosclerosis.

In contrast to our results, Arends et al.[30] and Erten et al.[31] have shown non-significant differences between levels of 25(OH)D3 and CIMT in AS patients (p=0.787). Gupta et al.[23] have also reported a non-significant relationship between CIMT and disease duration (p>0.05) or disease activity (p>0.05).

We have documented that serum levels of 25(OH)D3 negatively correlated with disease activity scores regarding BASDAI, BASMI and BASFI (r=-0.69, r=-0.41, r=-0.71, respectively) in AS patients. Also, serum levels of 25(OH)D3 had a negative correlation with lipid profile regarding cholesterol, LDL and TG (r=-0.45, r=-0.77, r=-0.59, respectively) while being positively correlated with HDL (r=0.40) and negatively correlated with VAS (r=-0.62) (Table 3).

In accordance to our results, Reynolds and Bruce[8] have reported that 25(OH)D3 deficiency is associated with CVD risk factors; therefore, it may indirectly predispose to atherosclerosis due to its positive relationship with HDL levels. Klingberg et al.,[32] Kienreich et al.[33] and Urruticoechea-Arana et al.,[34] have also revealed a positive correlation between 25(OH)D3 levels and HDL.

Moreover, Kunadian et al.[35] and Cai et al.[36] have also reported a significant relationship between low 25(OH)D3 levels and disease activity scores regarding BASDAI (p=0.06) in patients with AS. In contrast, Maki et al.[37] have found non-significant differences between serum levels of 25(OH)D3 and lipid profile (p=0.78). Kamycheva et al.[9] and Deleskog et al.[10] have also reported a non-significant relationship between low 25(OH)D3 levels and disease activity scores (p=0.37).

The limitations of this study are the small sample size and the cross-sectional design.

In conclusion, the prevalence of carotid atherosclerosis in AS patients compared to our healthy population was associated with high disease activity and functional limitations. In AS patients, 25(OH)D3 deficiency is a risk factor for atherosclerosis.

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