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THE RELATIONSHIP BETWEEN SMOKING AND ANKYLOSING SPONDYLITIS ON SOME SERUM BIOMARKERS

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Abstract
Ankylosing spondylitis (AS) is arthritis that affects the spine cause inflammatory stiffening of it. The disease affects both cartilaginous joints of the spine and the sacroiliac joints. Ossification of ligaments and spinal fusion with a typical stooped posture has been shown when disease was developed. Tobacco smoking consist of a complex mixture of various agents had many toxic effects so it has a negative influence on spinal activity, functional ability and mobility in AS. The pathogenesis of AS is imprecise and possible of several pro-inflammatory cytokine formation is leading to many pathogenic consequences. The liver responsible for releasing several types of acute phase proteins (APP) containing the fetuin- A (FA) as counter-regulatory mechanism. Vitamin D (VD) is very important for bone health, and has also been linked with immune function and protection against cancer. This study was designed to clarify the impact tobacco smoking on serum fetuin A, vitamin D and tumor necrosis factor alpha (TNF-α) levels in Iraqi male with ankylosing spondylitis. Cross- sectional study was done in Baghdad Teaching Hospital/ Medical City from April to August 2016. The number of both AS and volunteers are seventy-eight; their mean age were (36.53 ± 8.46) and (33.04 ± 9.74) years respectively. All of them were classified into four groups depending on healthy status and tobacco smoke. Blood sample was drawn from vein of each participant to determine the above serum biomarkers by ELISA. The result showed not significant variations in measuring hemoglobin (HGB) but significant increase of white blood cells (WBCs), platelets count and erythrocyte sedimentation rate (ESR){P values list > 0.05, < 0.05, < 0.05 and <0.01 respectively}. There is high significant decrease in serum VD and FA (P value= 0.00 and P value< 0.01) with increasing of TNF-α (P value <0.01) among four different groups. From the result be accomplish that fetuin A with vitamin D and TNF-α play essential role in prognosis and etiology of AS whether smoke cigarette or not. This study be the first done in Iraqi AS male to assess the effect of smoking on selected serum biomarkers especially fetuin A.

Keywords: Ankylosing spondylitis, vitamin D, fetuin- A, tumor necrosis factor alpha, cigarette smoking

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INTRODUCTION

The condition that generally affect spine was named ankylosing spondylitis (AS) leading to inflammation of many joints such as neck, back and pelvis, causing pain and stiffness as well as the sacroiliac joints are commonly affected. These joints bond the base of sacrum to pelvis but further joints (hips and shoulders) can also be involved. The eyes, skin, bowel and lungs also affect by AS. The ages between 15- 45 years are typically to start this condition. (1) The pathogenesis of AS is multifactorial, as in many autoimmune diseases, and based on endogenous factors, such as the genetic influences of HLA-B27 and exogenous factors. (2) Smoking has a recognized influence on the disease course and is associated with poor long-term outcome in AS patients (3). In last ten years, several studies done to clarify the role of macronutrients and micronutrients foods in the progression and development of chronic diseases (4, 5).

Active vitamin D 1, 25 $(OH)_2$ D excites absorption of intestinal calcium (6). When vitamin D deficient this affect the absorption of exogenous calcium and phosphorus intake (7, 8).

Fetuin-A is divergently controlled by different pro-inflammatory mediators (TNF, IFN-γ, and HMGB1), and functions as a positive or negative acute phase protein in injury and infection (9).

The aim of this study is to assess serum level of fetuin A as a predictive biomarker in Iraqi smoker male with AS in addition to tumor necrosis factor alpha (TNF-α) and vitamin D (VD). From our knowledge: no one measure serum fetuin A in AS.

SUBJECTS, MATERIALS AND METHODS:

Subjects include male with AS and control without AS (C). The study was carried out over 4 month's period in 2016 at Medical City / Baghdad Teaching Hospital. The mean age ± standard deviation (SD) equal (36.53 ± 8.46) for AS and (33.04 ± 9.74) for control and Body mass index within normal level for all of them {BMI = Weight (kg) / Height (m^2)} (10). The diagnosis of AS male was done by rheumatologist according to Bath Ankylosing Spondylitis Functional Index (BASFI) (11) and Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) (12). Many cases were excluded: newly diagnosed, chronic diseases, rheumatic diseases, endocrine and metabolic disorders. The estimation of the kidney function tests (KFTs) blood urea and serum creatinine (13,14) and liver function tests (LFTs) alanine& aspartate transaminase (15); the data refer that both KFT& LFT were normal for smokers and non- smokers P values registered not significant among all groups. Hemoglobin (16), WBCs and platelets count (17) were measured by automated (Architect C8000, Cell- Dyn / Abbott/ USA) and ESR (18) Westergren method assessed by (Microsed system auto analyzer/ Simians/ Germany). The smoker participants used one packet of tobacco daily. Total participant (male) mentioned in (figure 1) were divided into two major groups followed by four minor groups.

Group A: AS **Group B: C**

Group 1: Smoker AS
Group 2: Non- smoker AS
Group 3: Smoker C
Group 4: Non-smoker C

In this study venous blood samples were drawn from each participant. Blood sample saved in EDTA tube for assessing both complete blood picture and erythrocyte sedimentation rate (ESR). Part of whole blood centrifuged to obtain serum for detection (Vitamin D, fetuin A and TNF-α)/ Cal biotech / USA, Shanchai Yehua Biological Technology Co., Ltd / China respectively ELISA kits. The percentage of each group along with smoking habit illustrated in (figure2).

This revision was approved by College of Pharmacy/ University of Baghdad ethical committee and written permissions were obtained from all AS participants.

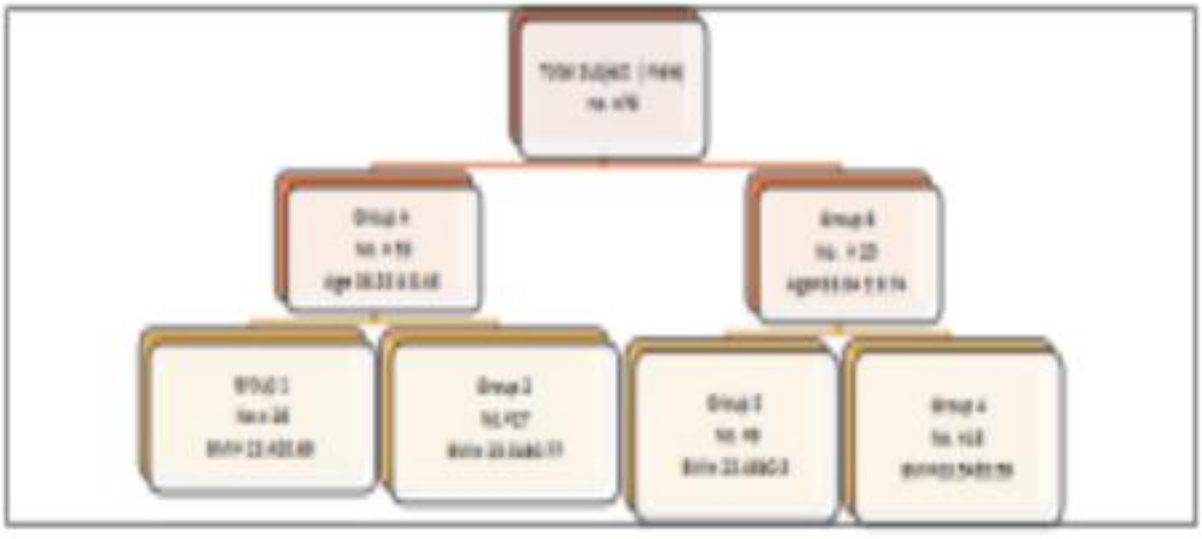

Figure 1. Demographic distribution of male with Ankylosing Spondylitis (AS) and control (C)

The measurement of hemoglobin (HGB), White blood cells (WBCs), platelets count and erythrocyte sedimentation rate (ESR) mentioned in table 1. Significant results related with WBCs and platelets counts and highly significant for ESR. (P values= 0.025, 0.018 and 0.00).

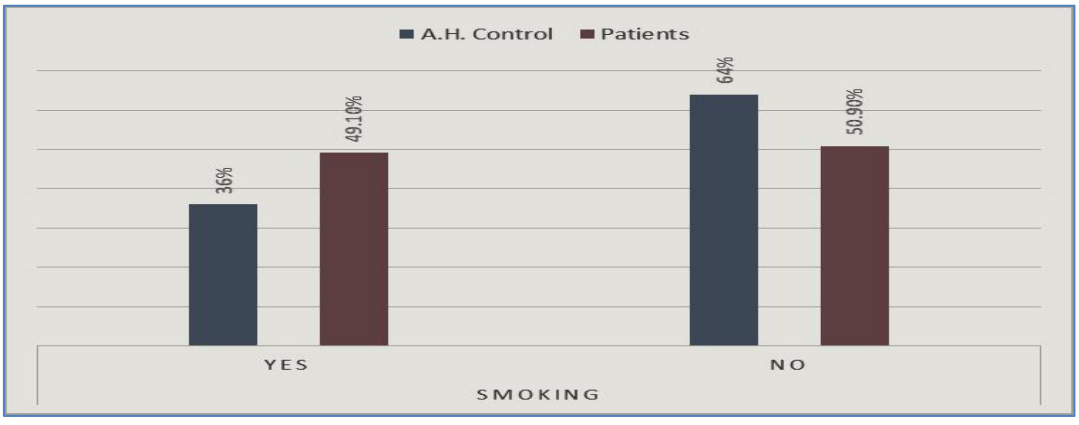

Figure 2. The percentage distribution of AS and control depending on their habit of smoking tobacco

Table 1. Hematological analysis of patients and controls

Studied groups	N	Mean	Std. Deviation	Std. Error	ANOVA test (P-value)

HGB g/dl	G1	26	14.142	1.3234	.2595	P=0.824 Non sign. (P>0.05)
	G2	27	14.122	1.5839	.3048	
	G3	9	14.300	.6245	.2082	
	G4	16	14.475	.8729	.2182	
WBCs count x103/ µl	G1	26	8.901*	1.7272	.3387	P=0.025 Sign. (P<0.05)
	G2	27	8.189^	1.9565	.3765	
	G3	9	8.748*	1.2061	.4020	
	G4	16	7.292^	1.2327	.3082	
Platelets count x103/ µl	G1	26	268.77*	60.135	11.793	P=0.018 Sign. (P<0.05)
	G2	27	270.15^	82.896	15.953	
	G3	9	205.89*	39.094	13.031	
	G4	16	225.00^	54.096	13.524	
ESR mm/ Hr	G1	26	17.38*	9.381	1.840	P=0.00 Highly sign. (P<0.01)
	G2	27	26.44^	23.837	4.587	
	G3	9	5.67*	3.000	1.000	
	G4	16	7.50^	3.830	.957	

(Table 2) demonstrate serum vitamin D, fetuin A and TNF-α level. P value was highly significant for all above markers (P values= 0.00, 0.005, 0.001). Correlation among different groups mentioned in table 3. Vitamin D showed high significant correlation between smoker AS (G1) and both smoker & non- smoker C (P value =0.00) also non- smoker AS correlate highly significant with (G3& G4) (P value= 0.00). Fetuin A showed high significant correlation among AS groups (G1, G2) and smoker C (G3), P value= 0.001. TNF- α result showed high significant correlation between AS & C (smoker or non- smoker), P values= 0.04, 0.013, 0.003 and 0.00.

Table 2 he serum levels of vitamin D, Fetuin- A and TNF- α in four groups

Studied groups		N	Mean	Std. Deviation	Std. Error	ANOVA test (P-value)
Vitamin D3 (ng/ml)	G1	26	6.98*	8.14	1.59	P=0.00 Highly sign. (P<0.01)
	G2	27	10.79^	9.52	1.83	
	G3	9	63.01*	41.80	13.93	
	G4	16	59.42^	40.60	10.15	
Fetuin - A (ng/ml)	G1	26	290.14*	82.71	16.22	P=0.005 Highly sign. (P<0.01)
	G2	27	304.0^	131.02	25.21	
	G3	9	959.01*	131.83	439.44	
	G4	16	461.20^	484.24	121.06	
TNF-α (pg/ml)	G1	26	131.9*	69.07	13.54	P=0.001 Highly sign. (P<0.01)
	G2	27	177.38^	174.95	33.66	
	G3	9	42.2*	25.40	8.46	
	G4	16	41.26^	28.79	7.19	

Table 3. Pearson correlation of serum biomarkers among studied groups.

Studied groups			LSD test (P-value)
Vitamin D3	G1	G2	P=0.566 NS
		G3	P=0.00 HS
		G4	P=0.00 HS
	G2	G3	P=0.00 HS
		G4	P=0.00 HS
	G3	G4	P=0.721 NS
Fetuin - A	G1	G2	P=0.919 NS
		G3	P=0.001 HS
		G4	P=0.297 NS
	G2	G3	P=0.001 HS
		G4	P=0.316 NS
	G3	G4	P=0.018 S
TNF- alpha	G1	G2	P=0.145 NS
		G3	P=0.042 S
		G4	P=0.013 S
	G2	G3	P=0.003 HS
		G4	P=0.00 HS
	G3	G4	P=0.984 NS

DISCUSSION

Ankylosing spondylitis (AS) is one kind of rheumatic diseases with prolonged autoimmune inflammation, had impact the axial skeleton in addition to extra-articular features and peripheral arthritis. The mean age of AS in this study were (36.53 ± 8.46) years in concomitant with previous revisions (19-21). Rodgman & Perfetti mentioned different types of substances in tobacco smoke (22) such as neutral gases, carbon and nitrogen oxides, amides, carboxylic acids, lactones, esters, aldehydes …etc. Hukkanen *et al.*, 2005 prescribed that nicotine of tobacco smoke had addictive character (23).

Level of hemoglobin (HGB) list normal in all groups while (WBCs, platelets) count and ESR observed significant increased between diseased and control group in the presence of tobacco smoke effect; for this reason smoking related with enhancing rheumatic disease risky such as RA leading to rise disease activity and a reduced anti-TNF therapy response (24). In 2006 Costenbader and Karlson explain the impact of smoking on disease autoimmunity at time of tissue damage the formation of cellular debris and starting apoptosis with auto-antigen overloading end to recruit autoimmune response, inflections in immunity (cellular and humoral) then induction of autoimmune processes (25). In this study ESR was in parallel with Kaut *et al*. finding it records not significant increase when studied in both gender (26); while hematological analysis, serum creatinine and alanine aminotransferase were in concomitant with W. Gaber *et al*. (27).

Vitamin D (VD) had immune-modulatory character and anti-inflammatory behavior and linking with AS disease activity unclear. The results show highly significant deceasing in VD in NAS if compare with NSC groups. The explanation varies according to previous studies some of them in concomitant with current study and other opposite it. In two different studies by Erten *et al* and Hmamouchi *et al* demonstrated the relation between AS disease activity and deficiency of vitamin D while Klingberg *et al* and Arends *et al* showed no effects. Other studies clarified the deficiency of vitamin D may enhance AS risky (28-33). The effect of tobacco smoke and VD shown in Aboraia *et al* studied the role of cigarette smoke metabolite (tetralones) that depress activity of CYP27A1(34). Our results agree with Jaaskelainen *et al* findings about smoker men had low serum 25-hydroxy vitamin D than non- smokers (35).

Fetuin A is glycoprotein of alpha-2-HS type detected in the plasma of fetus; with the progression of fetus life this plasma protein distributed in GIT, kidney, liver… According to injury or infection response fetuin A been synthesized principally by liver in adult life (36-39).

In current study: fetuin A records high significant decreasing of serum level in SAS, NAS than in SC, NC groups respectively in addition to high significant increase in ESR (table 1) so this finding is in the line of recent study by Papichev et al on rheumatoid arthritis women (RA) and old study by Sato et al results of when studied fetuin A in (RA) also in concomitant with K. Cagli et al. results when measuring serum fetuin A in rheumatic mitral valve disease and Saroha et al. reported lower serum fetuin-A levels in RA compared to controls (40-43).

This study is in parallel with Turkish study**:** Fetuin A serum concentration decreased in AS significantly with increased C-RP and not significant with ESR in both gender (44) also Oncu et al. previous work on familial Mediterranean fever showed the rising ESR, CRP WBCs count with lowering fetuin A as negative acute phase protein (45).

Poddubnyy et al demonstrated the relation between tobacco smoke and immune system through increasing of B and T

lymphocytes auto-reactivity, enhancing pro- inflammatory markers production interleukins, TNF-α, total neutrophils some matrix metalloproteinase so leading to stimulating free radical's synthesis causing oxidative stress (46). This finding is parallel with the present study in estimating TNF-α increase significantly when compare SAS with NAS groups. The AS is a disease of inflammatory nature, could increase the production of pro-inflammatory cytokines, interleukins (ILs) and TNF-α (47, 48).

CONCLUSION

From the results of this study it has been achieved that combination of fetuin A and vitamin D are good predictors for prognosis and etiology of AS in addition to advice the patients to cease tobacco smoke (cigarette, cigar and hookah) in order to reduced harmful effects. Finally: to the best of knowledge this trial is the first study in AS Iraqi patients to seem the association of cigarette smoking with serum fetuin A, vitamin D level and hematological analysis.

REFERENCES

1- Khan MA. Spondyloarthropathies. Rheum Dis Clin North Am 1992; 18(1): 1-276.
2- Khan MA, Van der Linden SM. Anklosing spondylitis and other Spondyloarthropathies, Rheum Dis Clin North AM 1990:16(3): ss1-79.
3- Bergen AW, Caporaso: N Cigarette Smoking, Journal of the National Cancer Institute, 1999; Vol. 91, No. 16, 1365- 75
4- Wolf AD, Pfleger B. Burden of Major Musculoskeletal Conditions. Policy and Practice. Special Theme-Bone and Joint Decade 2000- 2010. Bulletin of the World Health Organization 2003; 81: 646-56.
5- Braun J, Van den Berg R, Baraliakos X *et al.*: 2010 update of the ASAS/EULAR recommendations for the management of ankylosing spondylitis. Ann Rheum Dis 2011; 70: 896-904.
6- Moyad MA: Vitamin D: A Rapid Review; DISCLOSURES Dermatology Nursing. 2009; 21(1).
7- Lips P, Hosking D, Lippuner K, *et al.*: The prevalence of vitamin D inadequacy amongst women with osteoporosis: An international epidemiological investigation. J Intern Med. 2006; 260:245–54
8- Nair R and Maseeh A: Vitamin D: The "sunshine" vitamin. J Pharmacol Pharmacother, 2012; 3(2): 118–126
9- Wang H and Andrew E. Sama AE: Anti-inflammatory role of Fetuin- A in Injury and Infection; Curr Mol Med. 2012; 12(5): 625–63.
10- Daniels, S.: The use of BMI in the clinical setting. Pediatrics,2009; 124(Suppl1), S35-S41
11- Calin A, Garrett S, Whitelock H, *et al*.: A new approach to defining functional ability in ankylosing spondylitis: the development of the Bath Ankylosing Spondylitis Functional Index. J Rheumatol,1994; 21: 2281-85
12- Sieper J, Rudwaleit M, Baraliakos X, *et al*. The Assessment of Spondyloarthritis international Society (ASAS) handbook: a guide to assess spondyloarthritis. Ann Reum Dis, 2009; 68(2):1-44.
13- Patton CJ, and Crouch SR. Spectrophotometric and kinetic investigation of Berthelot reaction for the determination of ammonia. Anal Chem, 1977; 49:464-469.
14- Bartels H, and Bohmer M. A kinetic colorimetric method for determination of creatinine. Clin Chem Acta, 1972; 37:193.
15- Reitman S, and Frankel S. A colorimetric method of determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Am J Clin Path, 1957; 28: 56.
16- Balasubramaniam P, Malathi A, and Viswanathan C. A simple technique for hemoglobin estimation to screen for anemia. Indian J Physiol Pharmacol 1992; 36(3):213-214.
17- Wallestein RO. Laboratory evaluation of anemia. West J Med; 1987; 146: 433.
18- Jou JM, Lewis SM, Briggs C *et al*.: Review of the measurement of the erythrocyte sedimentation rate. Int J Lab Hematol 2011; 33(2):125-32
19- Mahmood F and Helliwell P: Ankylosing spondylitis: A review. EMJ; 2017;2[4]:134-139
20- Lopes S and Mesquita C: Ankylosing Spondylitis Intervention: What are the Options in Clinical Advances in Spondylitis; Chapter 3, 2017,1-16
21- Golder V and Schachna L: Ankylosing spondylitis: an update. Australian Family Physician; 2013, Vol. 42, No. 11, 780-784
22- Rodgman A and Perfetti T: The Chemical Components of Tobacco and Tobacco Smoke, Second Edition Book · 2013; CRC Press, xxvii- cxxviii
23- Hukkanen J, Jacob P, Benowitz NL: Metabolism and disposition kinetics of nicotine. Pharmacol Rev. 2005; 57(1):79-115.
24- Harel-Meir M, Sherer Y and Shoenfeld Y: Tobacco smoking and autoimmune rheumatic diseases; Nature Clinical Practice Rheumatology, 2007 VOL 3 NO 12.
25- Costenbader KH and Karlson EW: Cigarette smoking and autoimmune disease: what can we learn from epidemiology? Lupus, 2006; 15**:** 737–745.
26- Kaut IK, Abourazzak FE, Jamila E *et al.:* Axial Spondyloarthritis and Cigarette Smoking the Open Rheumatology Journal, 2017, 11, 53-61
27- Gaber W, Hassen AS, Abouleyoun II *et al*.: Impact of smoking on disease outcome in ankylosing spondylitis patients; 2015; Elsevier;1-5
28- Erten S, Kucuksahin O, Sahin A, *et al*.: Decreased plasma vitamin D levels in patients with undifferentiated spondyloarthritis and ankylosing spondylitis. Internal Medicine 2013; 52:339-344
29- Hmamouchi I, Allali F, Handaoui BE *et al*.: The relation between disease activity, vitamin D levels and bone mineral density in men patients with ankylosing spondylitis. Rheumatology Reports 2013; 5: 1.
30- Klingberg E, Olerod G, Hammarsten O *et al.:* The vitamin D status in ankylosing spondylitis in relation to intestinal inflammation, disease activity, and bone health: a cross-sectional study," Osteoporosis International 2016; 27: 2027-2033.
31- Arends S, Spoorenberg A, Bruyn G. *et al*.: The relation between bone mineral density, bone turnover markers, and vitamin D status in ankylosing spondylitis patients with active disease: a cross-sectional analysis. Osteoporosis International 2011; 22: 1431-1439.
32- Zhao S, Duffield SJ, Moots RJ *et al*.: Systematic review of association between vitamin D levels and susceptibility and disease activity of ankylosing spondylitis. Rheumatology 2014; 53: 1595-1603.
33- Ramagopalan S. V, Goldacre R, Disanto G *et al*.: Hospital admissions for vitamin D related conditions and subsequent immune-mediated disease: record linkage studies. BMC Medicine 2013; 11: 171
34- Aboraia AS, Makowski B, Bahja A, *et al*.: Synthesis and CYP24A1 inhibitory activity of (E)-2-(2-substituted benzylidene) - and 2-(2-substituted benzyl)-6-methoxytetralones. Eur J Med Chem, 2010; 45: 4427-4434.
35- Jaaskelainen T, Knekt P, Marniemi J *et al*.: Vitamin D status is associated with socio-demographic factors, lifestyle and metabolic health. Eur J Nutr, 2013; 52: 513-525.

36- Christie DL, Dziegielewska KM, Hill RM *et al*.: Fetuin: the bovine homologue of human alpha 2HS glycoprotein. FEBS Lett. 1987; 214(1):45–49.
37- Pedersen KO. Fetuin, a new globin isolated from serum. Nature. 1944; 154:575–570.
38- Dziegielewska KM, Daikuhara Y, Ohnishi T et al.: Fetuin in the developing neo cortex of the rat: distribution and origin. J Comp Neurol. 2000; 423(3):373–388.
39- Kitchener PD, Dziegielewska KM, Hutton EJ *et al*.: Fetuin in neurons of the retina and cerebellum during fetal and postnatal development of the rat. Int J Dev Neuro Sci. 1999; 17(1):21–30.
40- Papichev E, Sivordova L, Polyakova J *et al*.: Fetuin -A: clinical and laboratory associations in women with rheumatoid arthritis; Ann Rheum Dis: /annrheumdis-2018-eular;1228
41- Sato H, Kazama JJ, Wada Y *et al*.: Decreased levels of circulating alpha2-Heremans-Schmid glycoprotein/Fetuin-A (AHSG) in patients with rheumatoid arthritis. Intern Med; 2007; 46: 1685-91.
42- Cagli K, Basar N, Cagli K, *et al*.: Association of serum fetuin-A with valvular calcium concentration in rheumatic mitral valve disease. *J Heart Valve Dis*. 2010; 19:636–64
43- Saroha A, Kumar S, Chatterjee BP *et al*.: Jacalin bound plasma O-glycoproteome and reduced sialylation of alpha 2-HS glycoprotein (A2HSG) in rheumatoid arthritis patients. PLoS One 2012; 7: e46374
44- GÖKMEN F, AKBAL A, GÜNEŞ F *et al*.: Serum Fetuin-A level in patients with ankylosing spondylitis and its relationship with clinical parameters, Arch Rheumatol. 2015;30(1):1-5
45- Oncu K, Yazgan Y, Tanoglu A *et al*.: Can serum fetuin-A be regarded as an inflammatory marker among patients with familial Mediterranean fever? Dig Dis Sci 2013; 58: 3212-7.
46- Poddubnyy D, Haibel H, Listing J, et al. Baseline radiographic damage, elevated acute-phase reactant levels, and cigarette smoking status predict spinal radiographic progression in early axial spondylarthritis. Arthritis Rheum 2012; 64:1388-98.
47- Ombrellino M, Wang H, Yang H *et al*.: Fetuin, a negative acute phase protein, attenuates TNF synthesis and the innate inflammatory response to carrageenan. Shock. 2001; 15(3):181–185.
48- Wang H and Sama AE: Anti-inflammatory role of Fetuin-A in Injury and Infection, Curr Mol Med. 2012 June 1; 12(5): 625–633.