Seroprevalence of anti-mannose binding lectin autoantibodies in patients with rheumatoid arthritis in Sana'a city- Yemen

Abstract

Rheumatoid arthritis (RA) characterized by synovial inflammation and destruction of cartilage and bone. Until now there is no single test that diagnoses RA, however, several blood tests may suggest the presence of this disease. RA is associated with the presence of a number of autoantibodies as such as rheumatoid factor (RF), anti-cyclic citrullinated peptide (ACPA) and anti-mannose binding lectin (anti-MBL).

This studyaimed firstly to investigate the presence of anti-MBL autoantibodies in the sera of RA patients and healthy controls and secondly to determine the diagnostic value of this marker in comparison with the classical RF, C- reactive protein (CRP) and ACPAamong RA cases.

This case-control study was conducted at four health establishments; two public (Al-Thawra Modern General Hospital and National Center of Central Public Health Laboratories) and two private (University of Science and Technology Hospital and Aulqi Specialized Medical Laboratories) in Sana'a city.

Ninety-four individuals were enrolled in this study. Forty-seven persons were clinically diagnosed to have RA by a rheumatologist and 47 healthy subjects without RA were used as controls. Sera were separated and tested for presence of serum anti-MBL autoantibodies, ACPA, RF and CRP by a commercially available enzyme linked immunosorbent assay (ELISA) and latex agglutination technique.

Study results showed that the mean±SD for the levels of serum anti-MBL autoantibodies among RA cases were 394±243 ng/ml which were significantly higher than that recorded among healthy controls (217±173 ng/ml). The levels of serum anti-MBL autoantibodies were associated with positive RF and CRP tests (p=.02 & .007 respectively), but not with positive ACPA test (p=.42).

The result of this study showed higher levels of serum anti-MBL autoantibodies among RA cases comparing with the healthy controlsand reveal an association with positive results for RF and CRP, but not with ACPA.Therefore, the anti-MBL antibody levels may associated with systemic autoimmune diseases and might not exclusive to RA.

Key words

Rheumatoid arthritis; anti-MBL autoantibodies; ACPA; RF,CRP

Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disease that affects 0.5%–1% of the worldwide population. It is characterized by chronic and erosive polyarthritis which in the majority of patients leads to partial disability or to permanent handicap. The exact etiology of RA is unknownbut is believed to be influenced by genetic, environmental, hormonal, immunologic, and infectious factors[1,2].

The diagnosis of RA based onphysical exam, radiographic & laboratory tests. Unfortunately, there is no single test can confirm the diagnosis of RA. The blood tests,

erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) are only markers of inflammation. RA is characteristically associatedwith the presence of many different autoantibodies directed against multiple autoantigens. The first discovered autoantibody in RAwas rheumatoid factor (RF).It was found to react with theFc portion of IgG antibodies. Typically, RF is of IgM isotype, but IgG and IgA may also occur. RF is found in 60–80% of RA patients and thus is fairly sensitive in the RA diagnosis. However, the specificity of RF as a diagnostic marker for RA is low since it is frequently detected in many other conditions such as connective tissue diseases[3, 4].Anti- citrullinated peptide antibody (ACPA) target peptides that are post-translationally modified by conversion of arginine to citrulline, occurs primarily in patients of RA and demonstrate a much higher specificity of 95.9% for RA.In addition, ACPA seem to be a better marker of poor prognostic features of progressive joint destruction[5, 6].Mannose-binding lectin (MBL) is a liver-derived acute phase protein. This C type lectin has specific binding affinity to mannose and N-acetyl glucoseamine which structurally homologus to C1q, the component of classical complement pathway. When MBL binds to the specific carbohydrate, its associated serine proteases (MBL associated serine proteases "MSAPs") become activated, leading to activation of lectin pathwayof the complement system [7-10].

MBL has an important role in provoking an inflammatory response and consequently has been well associated with the pathogenesis of different infectious and autoimmune diseases[11,13]. It has been shown that MBL binds both dimeric and polymeric IgA & activates complement[14]. Moreover, previous studies demonstrated that MBL can bind agalactosyl IgG& IgM including IgM RF complexes in RA patients, leading to generation of an inflammatory response[13, 15].The presence of autoantibodies against MBL in serum as well as in synovial fluid from several RA patients has been reported by many studies[16- 19], and they demonstrated that the anti-MBL decrease the functional activity of MBL in patients in SLE.This studyaimed firstly to investigate the presence of anti-MBL autoantibodies in the sera of patients with RA and healthy controls and secondly to determine the diagnostic value of this marker in comparison with the classical RF, CRP and ACPAamong RA cases.

Subjects and Methods

Subjects

The type of study was a case-control study which was carried out during a period from May, 2015 to May, 2016at Al-thawra Modern General Hospital,University of Science and Technology Hospital, National Center of Central Public Health Laboratories, and Aulqi Specialized Medical Laboratories in Sana'a city.A total number of ninety-four Yemeni participants were investigated for the presence of anti-MBL autoantibodies in their sera. Forty-seven cases were clinically diagnosed with RA by a rheumatologist according to the ACR criteria [20].In addition, forty-seven healthy individuals without RA were enrolled as controls. A full history was taken from each personand recorded in a predesigned questionnaire.

Five milliliter venous blood was collected from each participant into plain vacationer tubes. The sample was allowed to clot at room temperature and centrifuged at 3500 rpm for five minutes. Serum was then separated into eppendrof tubes and stored at -20 °C till tested.

Autoantibody tests

Presence of anti-MBL autoantibodies in the serum of each participant was tested using ELISA kit (Uscn, Life Science Inc, USA) and the presence of ACPA was also determined by ELISA (INOVA diagnostic kits, San Diego, CA-USA). RF and CRP were measured by latex tests (Vitro Scient Co, Egypt).Cutoff was calculated from the mean + 2SD of healthy controls which equals to 390 ng/ml

Statistical analysis

Statistical analysis was performed by using the Epi Info version 6 program (CDC, Atlanta, USA) for statistical significance.

Results

Out of the 47 cases with RA, females represented 81%, while males represented 19% with a mean age 43.3±14.8 years and 49% of ages were in group ≥50 years. In addition, out of the 47 healthy controls, females represented 83%, while males represented 17% with a mean age 43.2±14.9 years and 47% of ages were in group ≥50 years,(Table 1).

The levels of serum anti-MBL autoantibodies were significantly increased among RA cases, in which the mean ± SD of anti-MBL levels among RA cases was 394± 243 ng/ml higher than the mean ± SD of healthy controls which was 217± 173 ng/ml with a statistical significance (p=0.001), (Table 2). As regard the association of anti-MBL with other serological markers. The levels of serum anti-MBL autoantibodies were significantly higher among cases with RF and CRP positive than negative (418.4±232, 418.8±229 ng/ml,respectively) and the other statistical variables as median, mode and ranges were also higher for cases than controls. On the other hand, the levels of anti-MBL were higher among cases with ACPA negative 415.4±208 ng/ml than positive 376.8±257 ng/ml (Table 3).

Discussion

At last years, a growing evidence showed the importance of innate immune system involving lectin pathway of complement activation, of which MBL play a crucial role [21-23]. The low level of MBL has been apparently associated with inflammatory autoimmune diseases such as RA, SLE and other related diseases, also the deficiency of MBL can enhance the risk of infection [21, 24, 25]. The present study aimed firstly to investigate the presence of anti-MBL autoantibodies in the sera of patients with RA and secondly to determine the diagnostic value of this marker in comparing with the classical RF, CRP and ACPA.

The results of this study showed an obvious measurableand a higher significant presence of anti-MBL autoantibodies in the sera of enrolled RA cases (mean±SD= 394 ± 243 ng/ml) as compared with healthy controls(mean±SD= 217 ± 173 ng/ml)(p= 0.001). This result was similar to that reported by Gupta *et al.*[26]in India which showed a detectable significant presence of anti-MBL autoantibodies in the sera of RA patients as compared to the controls. This study demonstrated that the anti-MBL were more often in RA patient sera and suggested that it have a diagnostic value for RA.

When considered the association of the levels of anti-MBL with the levels of RF and CRP in this patients,the anti-MBL autoantibody levels were significantlyassociated with the positive cases of these markers in which the mean±SD of anti-MBL levels equal to 418.4±232 &418.8±229 ng/ml, respectively. Our results were in agreeingwith study by

Di Muzio *et al.*[27], and disagree with study by Gupta *et al.* which reported that the levels of anti-MBL antibodies were still positive and high in negative cases of RF & CRP[26].

As regard ACPA,there was no association between positive ACPA and the levels of anti-MBL autoantibodies, in which the mean±SD of anti-MBL levels for ACPA positive cases was 376.8±257 ng/ml, and for negative ACPA were 415.4±208 ng/ml. To our knowledge, there is no previous study in the relation between anti-MBL levels and ACPA in RA patients that agreed or disagreed with our results.

Several studies reported that the anti-MBL autoantibodies found in in different systemic autoimmune diseases as SLE and RA. These studies demonstrated that anti-MBL autoantibodies play a pathogenic role in the development of autoimmunity [16-19, 28, 29]. Depend on the specificity of ACPA to RA[5, 6]and according to the association of anti-MBL autoantibody levelswith positivity of RF and CRP but not with ACPA in our results, we can have concluded that the anti-MBL antibody levels may associated with systemic autoimmune diseases and might not exclusive to RA.

Conclusion and recommendations

In our study, the levels of serum anti-MBL autoantibody were significantly increased in RA cases when compared with the healthy controls and a significant association was found between the levels of serum anti-MBL autoantibody, RF and CRP positive results, but no association with ACPA positive results.Further studies should be applied to detect the diagnostic value of serum anti-MBL autoantibody levelsin RA as well as the molecular mechanism of its interaction inRA.

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Table (1): The age and sex distribution of studied subjects for the levels of serum anti-MBL autoantibodies

Characteristics variable	Cases **n=47**		Controls **n=47**		Total **n=94**	
	No.	**%**	**No.**	**%**	**No.**	**%**
Sex						
female	38	81	39	83	77	81.9
male	9	19	8	17	17	18.1
Total	**47**	**100**	**47**	**100**	**94**	**100**
Age groups/ Years						
<20	5	10.6	4	8.5	9	9.6
20-29	7	14.9	10	21.3	17	18.1
30-39	5	10.6	5	10.6	10	10.6
40-49	7	14.9	6	13	13	13.8
≥ 50	23	49	22	47	45	47.9
Total	**47**	**100**	**47**	**100**	**94**	**100**
Mean/Years ±SD	43.3 ± 14.8		43.2 ± 14.9		43.2 ± 14.8	
Min./Years	16		16		16	
Max./Years	66		66		66	

Table (2): Titer of anti-MBL autoantibodies in both RA patients and the controls

Anti-MBL levels ng/ml	Cases **n=47**	Controls **n=47**	***p***
Mean± SD	394 ±243	217±173	<0.001
Min.	15	14	
Max.	899.6	781	

Table (3): The association between geometric mean ± SD, median, mode and range for the levels of serum anti-MBL autoantibodies and positive and negative inflammatory markers among RA cases

		Anti-MBL autoantibodies levels ng/ml				
Inflammatory markers		Mean ±SD	Median	Mode	Min.-Max.	***p***
Anti-CCP	Positive	376.8±257	318.5	597.5	15.2 – 894	0.42
	Negative	415.4±208	371	191	191- 781	
RF	Positive	418.4±232	419	597.5	29.5 – 894	0.02
	Negative	300.7±265	232	15.2	15.2-781	
CRP	Positive	418.8±229	419	597.5	29.4 – 894	0.007
	Negative	277.8±273	191	15.2	15.2 – 781	