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 unknown but is believed to be influenced by genetic, environmental, hormonal, immunologic, and infectious factors. The diagnosis of RA based on physical exam, radiographic and 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 associated with the presence of many different autoantibodies directed against multiple autoantigens. The first discovered autoantibody in RA was rheumatoid factor (RF). It was found to react with the Fc 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. 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. 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 homologous 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 pathway of the complement system.

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. It has been shown that MBL binds both dimeric and polymeric IgA and activates complement. Moreover, previous studies demonstrated that MBL can bind agalactosyl IgG and IgM including IgM RF complexes in RA patients, leading to generation of an inflammatory response. The presence of autoantibodies against MBL in serum as well as in synovial fluid from several RA patients has been reported by many studies, and they demonstrated that the anti-MBL decrease the functional activity of MBL in patients in SLE. This study aimed 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 ACPA among 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, 2016 at 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. In addition, forty-seven healthy individuals without RA were enrolled as controls. A full history was taken from each person and 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 eppendorf 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 (Usen, 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±217 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).

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 |
|--------------------------|------------|--------------|------------|
| Sex                      |            |              |            |
| female                   | 38         | 81           | 77         | 81.9 |
| male                     | 9          | 19           | 17         | 18.1 |
| Total                    | 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         |      |     |     |
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).

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            |       |

DISCUSSION
At last years, growing evidences showed the importance of innate immune system involving lectin pathway of complement activation, of which MBL play a crucial role. 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. The present study aimed firstly to investigate the presence of anti-MBL auto antibodies 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.

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

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

Several studies reported that the anti-MBL autoantibodies found 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. Depend on the specificity of ACPA to RA and according to the association of anti-MBL autoantibody levels with positivity of RF and CRP but not with ACPA in current 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 current 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. The results of this study showed an obvious measurable and 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., 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 significantly associated with the positive cases of these markers in which the mean±SD of anti-MBL levels equal to 418.4±232 and 418.8±229 ng/ml, respectively. Current study results were in agreeing with study by Di Muzio et al., 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 and CRP. 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 current results.

AUTHOR’S CONTRIBUTION
The manuscript was carried out, written, and approved in collaboration with all authors.

CONFLICT OF INTEREST
No conflict of interest associated with this work.

REFERENCES
1. McInnes IB, Schett G. The pathogenesis of rheumatoid arthritis. N Engl J Med 2011; 365:2205-19. https://doi.org/10.1056/NEJMra1004965
2. Isaaca JD. The changing face of rheumatoid arthritis: sustained remission for all? Nature Rev 2010; 10:605-11. https://doi.org/10.1136/ard.2009.117234
3. Waaler E. On the occurrence of a factor in human serum activating the specific agglutination of sheep red corpuscles. Acta Pathol Microbiol Scand 1940; 17:172-88. https://doi.org/10.1111/j.1600-0463.2007.apm.682.x

4. Aleluah, A.; Khudoyan, S. Therapeutic implications of autoantibodies in rheumatoid arthritis. RMD Open 2016; 2(1). https://doi.org/10.1136/rmdopen-2014-000009

5. Hayashi N, Kumagai S. Anti-cyclic citrullinated peptide antibodies and rheumatoid arthritis. Rinsho Byori. 2010; 58(5): 466-79. PMID: 20506046

6. Van der Linden MP, Van der Woude D, Iaon-Facсинay A, et al. value of anti-modified citrullinated vimentin and third generation anti-cyclic citrullinated peptide compared with second generation anti-cyclic citrullinated peptide and rheumatoid factor in predicting disease outcome in undifferentiated arthritis and rheumatoid arthritis. Arthritis Rheum. 2009; 60:2232-41. https://doi.org/10.1002/art.24716

7. Turner MW, Hamvas RM. Mannose-binding lectin: structure, function, genetics and disease association. Rev Immunogenet 2000; 2:305e22. PMID: 11256742

8. Ohha M, Okada M, Yamashina I, Kawasaki T. The mechanism of carbohydrate-mediated complement activation by the serum mannan-binding protein. J Biol Chem 1990; 265:1980e4.

9. Holmskov U, Malhotra R, Sim RB, Jensenius JC. Collectins: collagenous C-type lectins of the innate immune defense system. Immunol Today 1994; 15:67-74. https://doi.org/10.1016/0167-5699(94)90136-8

10. Petersen SV, Thiel S, Jensenius JC. The mannan-binding lectin pathway of complement activation: biology and disease association. Mol Immunol 2001; 38: 133-49.

11. Kilpatrick DC. Mannan-binding lectin: clinical significance and applications. Biochim Biophys Acta 2002; 1572:401e13. https://doi.org/10.1016/S0167-4781(02)00221-5

12. Eisen DP, Minchinton RM. Impact of mannose-binding lectin on susceptibility to infectious diseases. Clin Infect Dis 2003; 37:1496e505. https://doi.org/10.1086/379342

13. Malhotra R, Wormald MR, Rudd PM, Fischer PB, Dwek RA, Sim RB. Glycosylation changes of IgA associated with rheumatoid arthritis can activate complement via the mannose-binding protein. Nat Med 1995; 1:237e43. https://doi.org/10.1038/nn0395-237

14. Roos A, Bouwman LH, Munoz J, Zuiverloon T, Faber-Krol MC, Falaux-van den Houten FC, Klar-Mohamad N, Hack CE, Tilianus MG, Daha MR. Functional characterization of the lectin pathway of complement in human serum. Mol Immunol. 2003; 39: 655-68. https://doi.org/10.1016/S0168-1215(02)00859-6

15. Sato R, Matsushita M, Sato Y, Kasukawa R, Fujita T. Structures reactive with Mannose binding protein in sera of patients with rheumatoid arthritis. Fukushima J Med Sci 1997; 43:99-111

16. Seelen MA, Trouw LA, Van Der Hoorn JWA, et al. Autoantibodies against mannos-N binding lectin in systemic lupus erythematosus. Clin Exp Immunol 2003; 134: 335-43. https://doi.org/10.1046/j.1600-2249.2003.02274.x

17. Mok MY, Jack DL, Lau CS, et al. Antibodies to mannos-N binding lectin in patients with systemic lupus erythematosus. Lupus 2004; 13: 522-8. https://doi.org/10.1111/j.1600-2249.2004.02477.x

18. Takahashi R, Tsutsumi A, Ohtani H, et al. Anti-mannose binding lectin antibodies in sera of Japanese patients with systemic lupus erythematosus. Clin Exp Immunol 2004; 136: 585-90. https://doi.org/10.1111/j.1600-2249.2004.02477.x

19. Pradhan V, Surve P, Ghosh K. Mannose Binding Lectin (MBL) in Autoimmunity and its Role in Systemic Lupus Erythematosus. J Assoc Physicians India. 2010; 58:688-90.

20. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, Healey LA, Kaplan SR, Liang MH, Lurra HS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988; 31(3):315-24.

21. Heizeneder S, Seidel M, Forster-Waldl E, Heitger A. Mannan-binding lectin deficiency - Good news, bad news, doesn’t matter? Clin Immunol 2012; 142:22-38. https://doi.org/10.1016/j.clim.2011.11.002

22. Chen M, Daha MR, Kallenberg CG. The complement system in systemic autoimmune disease. J Autoimmun 2010; 34: 276-86. https://doi.org/10.1016/j.jaut.2009.11.014

23. Degn SE, Jensenius JC, Thiel S. Disease-causing mutations in genes of the complement system. Am J Hum Genet 2011; 88: 689-705. https://doi.org/10.1016/j.ajhg.2011.05.011

24. Summerfield JA. Clinical potential of mannose-binding lectin-replacement therapy. Biochem Soc Trans 2003; 31: 770-3. https://doi.org/10.1042/BST0310770

25. Chalmers JD, McHugh BJ, Doherty C, Smith MP, Govan JR, Kilpatrick DC, Hill AT. Mannose-binding lectin deficiency and disease severity in non-cystic fibrosis bronchiectasis: a prospective study. Lancet Respir Med 2013; 1(3):224-32. https://doi.org/10.1016/S2213-2600(13)70001-8

26. Gupta B, Raghav SK, Agrawal C, Chaturvedi VP, Das RH, Das HR. Anti-MBL autoantibodies in patients with rheumatoid arthritis: prevalence and clinical significance. J Autoimmun 2006; 27: 125-33. https://doi.org/10.1016/j.jaut.2006.07.002

27. Di Muzio G, Perricone C, Ballanti E, et al. Complement system and rheumatoid arthritis: relationships with autoantibodies, serological, clinical features, and anti-TNF treatment. Int J Immunopathol Pharmacol 2011; 24: 357-66. https://doi.org/10.1177/0891428311405821

28. Pradhan V, Mahant G, Anjali Rajadhyaksha A, et al. A study on anti-mannose binding lectin (anti-MBL) antibodies and serum MBL levels in Indian systemic lupus erythematosus patients. Rheumatol Int 2013; 33: 1533-9. https://doi.org/10.1007/s00029-012-2519-9

29. Shoefeld Y, Szpyer-Kravitz M, Witte T, et al. Autoantibodies against protective molecules-C1q, C-reactive protein, serum amyloid P, mannose-binding lectin, and apolipoprotein A1: prevalence in systemic lupus erythematosus. Ann N Y Acad Sci 2007; 1108: 227-39. https://doi.org/10.1196/annals.1422.025