Association of ANA seropositivity with RF, CRP, Brucellosis test in patients with SLE, a compression between immunofluorescence technique and latex agglutination

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Objectives The present study aims to inspect the connection between patients with SLE and RF, CRP, Brucellosis and compression between immunofluorescence technique and latex agglutination method to detect ANA.
Methods A total of 90 serum sample collected from patients with symptoms and signs of SLE those send to the immunity lab in Al-Hussein hospital. The immunofloriscent ANA test, SLE latex agglutination test, RF, CRP and Rose Bengal tests were done to all these samples.
Results The results show that there was significant difference between males (16%) and females (84%) patients with sign and symptom of SLE, while there was no significant difference in CRP, RF, in patients with SLE positive (50%,17%) and SLE negative patients (48.5%,17%), continuously Rose Bengal test is higher in SLE negative patients than in SLE positive patients (15.2%, 8.3%). Finally, there is significant difference in diagnosis of SLE disease between IF technique and latex agglutination test (41.6%, 100%).
Conclusions Latex agglutination test for ANA detection is reliable, specific but less sensitive test when compared to SLE IF test. There are significance differences in Rose Bengal test in SLE negative and in SLE positive patient's, while there are non-significance difference in CRP, RF in SLE positive samples and in SLE negative samples.
Keywords ANA, latex, immunofluorescence, Rose Bengal test

Introduction

SLE (Systemic Lupus Erythematosus) was a multiple system of autoimmune disorder with a broad spectrum of clinical presentations.[1] There is a peak age of onset among young women between the late teens and early 40s and a female to male ratio of 9:1. Ethnic groups such as those with African or Asian ancestry were higher at risk of developing the disorder and it may be more severe compared to Caucasian patients. Systemic Lupus Erythematosus (SLE) was a chronic disease that may be life-threatening when major organs are affected but more commonly results in chronic debilitating ill health. There was no single cause of SLE has been identified though factors such as sunlight and drugs may precipitate the condition and there is a complex genetic basis.[2]

SLE was represented by immune dysregulation resulting in the production of autoantibodies such as ANA, that make generation of circulating immune complexes, and induce activation of the complement system.[3] A positive ANA result is consistent with SLE, and it is extremely rare for them to have a negative ANA.[4] The presence of ANA in SLE patients is both a diagnostic and a prognostic marker.[5]

Antinuclear antibody (ANA) tests were usually performed on patients' serum with various connective tissue diseases, especially in systemic lupus erythematosus (SLE), for diagnostic evidence, significance prognosis, and management of therapy. The higher titer level of ANA is found in active SLE and the presence of these antibodies was the second most common manifestations of SLE.

Immunofluorescence (IF) test was the best test of choice for screening for the presence of ANA since it detects 95–100% of active SLE cases. ANA has been well documented in many different diseases status as well as in healthy relatives of SLE patients. The incidence of a positive ANA has been varied with in each diseases.[6] Rheumatoid factors (RF) were antibodies that react with the individual's own immunoglobulin.[7] These antibodies were directed against the Fc region of the IgG molecule.

Rheumatoid Factor can be detected in the serum of the majority of patients with rheumatoid arthritis and is important for the diagnosis and prognosis of those patients with higher concentrations.[8] Rheumatoid factors was not specific disease that can present in a lower frequencies in multiple other autoimmune disorders, chronic inflammation and normal individuals.[9]

C-reactive protein (CRP) was special type of protein produced by liver organ has been only present during the episodes of acute inflammation. The most important role of CRP was that is interaction with complement system, which is one of the immunologic defense mechanisms of the body. C-reactive protein was a protein produced in the liver by the pro-inflammatory cytokines tumor necrosis factor alpha, interleukin-6, and interleukin-1B. Since CRP test was considered as general test, the positive CRP test may be indicative in any number of conditions; cancer, rheumatoid arthritis, tuberculosis, rheumatic fever, myocardial infarction, pneumococcal pneumonia, or SLE.[10.]

Malta fever is a bacterial disease caused by bacteria infection known as Brucella abortus.[11] Serological test confirm the diagnosis when symptoms are present. Rose Bengal test was usually the serological test that was recommended used for disease screening and in management of patients with finding a positive test even absence of symptoms, the positive test should be discussed with other inflammatory disease or other microbe infection, because Rose Bengal test is positive in 80% of patient and negative in 20% of patient.[12] Auto immunity and brucellosis is not clear and there are very limited publications about this interaction. Anti-nuclear

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antibody could be found in the serum of a brucellosis patient during the active stage of disease because the Brucella can produce autoantibody agents intercellular nucleus, the antibody produced by Brucella patient, is IgM that effected on joint and give false RF positive.[13]

Materials and Methods

A total of 90 serum sample collected from patient with symptoms & signs of SLE that send to the immunity lab in Al-Hussein hospital. The IFANA test was done to all these samples.[14.]

All 90 samples were detected for ANA latex (SLE latex test, Human), detection of Rose Bengal latex agglutination test. Meanwhile, RF and CRP latex agglutination tests were performed. The procedures done as indicated in the instruction leaflets of the kits.

Results

Ninety cases were investigated for IFANA test, 24 consecutive SLE patients who had a disease onset. The female/male ratio (76 females and 14 males).

Regarding RF, and as shown in Table 2, there is no significant difference between rheumatoid factor in patient with SLE 4/24 and in non-affected patients 11/66 at $P \leq 0.05$.

The result, as shown in Table 3, reveals no significance difference between CRP in patient with SLE 12/24 and there is no significant difference among non-affected patients too 34/66 at $P \leq 0.5$.

Data explained in Table 4 show significant difference between patient with SLE 2/24 (8%) and brucellosis positive test among non-affected patients 10/66 (15%) at $P \leq 0.05$.

This study include comparison between latex SLE test and to evaluate the real specificity and sensitivity of SLE Latex test

Table 1. **Distribution of SLE laboratory findings at presentation using IF ANA among male and female**

Number	If ANA +VE	If ANA −VE	Total
Male	2	12*	14
Female	22	54*	76
Total	24	66	90

(*) indicates statistically significant finding.

Table 2. **Relationship between SLE and Rheumatoid Factor in collected samples**

Number	RF +VE	RF −VE	Total
If ANA + VE	4	20	24
If ANA −VE	11	55	66
Total	15	75	90

All above results show statistically non-significant finding.

Table 3. **Relationship between SLE and CRP in collected samples**

Number	CRP +VE	CRP −VE	Total
If ANA +VE	12	12	24
If ANA –VE	32	34	66
Total	44	46	90

All above results show statistically non-significant finding.

Table 4. **Frequency of SLE and Brucellosis in collected samples**

Number	Rose +VE	Rose −VE	Total
If ANA +VE	2	22 *	24
If ANA –VE	10	56	66
Total	12	78	90

(*) indicates statistically significant finding.

Table 5. **Comparison between LATEX agglutination SLE test and IF ANA test used for diagnosis of SLE**

Number	If ANA +VE	If ANA −VE	Total
Latex ANA +VE	10	0	10
Latex ANA −VE	14	66	80
Total	24	66	90

(*) indicates statistically significant finding.

in comparision with immunofluorescent techniques for the diagnosis of SLE disease.

The result show that 10/24 (41.6%) from patient has positive IFANA are determined by SLE latex test as positive while 66/66(100%) of serum collected from IFANA test negative was negative by SLE latex test as explained in Table 5.

Discussion

Data of the current study reveals a significant difference between male and female in patients with SLE. Female was higher prevalence than male patients at $P \leq 0.05$.

ANA prevalence generally increased with age ($P = 0.01$) and was significantly higher in women than men (17.8% versus 9.6%; $P = 0.001$) Our findings of higher ANA prevalence in females, and older individuals are similar to several earlier reports.[15–17] The reason for the female predominance in autoimmune diseases is not completely understood; nevertheless, the finding of a similar pattern of female dominance in ANA production suggests that hormonal or other factors in females play a role in this process.[18,19]

Several authors reported prevalence of ANA in RA has been variable in several published series ranging from 10% to 70%.[20] The variability in prevalence is probably due to multiple factors including patient selection and technical factors such as type of substrate used in the test, characteristics of the fluorescent reagents, etc.

Previous studies concerning the relation between CRP and ANA seropositivity conclude that evaluated C-reactive protein (54.7%) patients,[21] and that there are major statistical limitations between CRP and ANA.[22]

Literature is rich with works linking brucellosis with articular complications. In regard to this association, a study of 96 cases in Kuwait detected that 26% incidence of osteoarticular complications in brucellosis in this study falls within the reported incidence of 11% to 56%.[7–10,12–16] The wide range of reported incidences might be explained not only by possible geographical variations but also by the lack of agreement on one definition for arthritis.[16,23,24] Whether this relation is a true complication of brucellosis infection or a mere of serologic cross reactivity is still in need for further confirmation studies to be proved.

This study confirms that the SLE latex agglutination test was highly specific (94%) but less sensitive (41.6%). The small sample recruited in this study may reveal confounding result

about this fact, thus, further studies with larger sample size is recommended to fix this finding.

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