ANA, RF and CRP in Patients with Some of Rheumatic Symptoms

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Abstract: -

One hundred three patients with Rheumatic Symptoms were included in this study and their sera tested for the presence of Antinuclear Antibody (ANA), Rheumatoid Factor (RF), C- Reactive Protein (CRP). All patients 36, 35% with Systemic Lupus Erythematosis (SLE) had +ve ANA, the remainder 67 patients were with Rheumatoid Arthritis (RA) (65%), the incidence of SLE was 4:1 female to male while of RA was 2:1. The highest titers of ANA, RF, CRP were in females. In the following age groups (10-14), (15-19) and (35-39) the incidence of SLE were in females only. No male or female with SLE in (40-44), (45-49) or (50-54).

Introduction

Immunodiagnostic or serodiagnostic studies of antigen – antibody reaction are used for diagnosis of infectious disease , autoimmune disease , immune allergies and neoplastic disease ;in such kind of studies blood serum is tested for antibodies against particular antigens[1]. Pathologically, autoimmune disorders are produced by autoantibodies that are directed against self antigens example, including systemic rheumatic diseases such as rheumatoid arthritis and systemic lupus erythematosis [2] .

An increasing number of autoantibodies can be detected. Production of some of these is a common and age- related phenomenon that may be exaggerated by chronic inflammation. Their mere presence, therefore often has low diagnostic specificity and little clinical relevance. If present in high concentration, however, their disease specificity often increases. It is therefore important to know how much antibodies are present (the titer or concentration in units) rather than just whether they are detectable . The correct choice and interpretation of these tests depend on detailed knowledge of the patient. Different detection and assay systems exist for many of these autoantibodies, and close liaison with the local immunology service required .[3,4] Anti Nuclear Antibody (ANA), Rheumatoid Factor (RF) and C-Reactive Protein (CRP) are among the three most frequently used as non specific markers of autoimmunity and can occur alone or in conjugation with autoantibody specificity in rheumatic diseases .[5]

Rheumatoid Factor (RF) :The blood of

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many persons with Rheumatoid Arthritis (RA) contains a macro-globulin-type antibodies called Rheumatoid Factor (RF). Evidence indicates that RFs are anti-gamma globulin-antibodies; however, until a specific antigen that produces RF is discovered, the exact nature of RF can only be speculated. Even more uncertain is the role that plays in RF. Although RF may cause or perpetuate the destructive changes associated with rheumatoid arthritis (RA), it may also beneficial to these changes or may even serve some beneficial purpose. RF is some times found in sera from patients with other diseases, even though RF incidence and values are higher in patients with (RA). This test is useful in the diagnosis of RA. It measures RF antibodies directed against the Fc fragment of IgG. These are usually IgM antibodies, but they may also be IgG or IgA[1].

C-Reactive Protein:- During many inflammatory, a specific abnormal protein named C-Reactive Protein (CRP) appears in the blood, this protein is virtually absent from the serum of healthy persons. CRP is one of the most sensitive acute phase reactants. Levels of CRP can increase dramatically (100 fold or more) after severe trauma, bacterial infection, inflammation, surgery or neoplastic proliferation. Measurements of CRP have been used historically to assess activity inflammatory disease, to detect infections after surgery, to detect transplant rejection, and to monitor these inflammatory processes [2].

Anti Nuclear Antibody (ANA):- Measurement of ANA in serum is the most commonly performed screening test for autoimmune bodies in patients suspected of having systemic rheumatic disease (SRD), SRD_s includes Systemic Lupus Erythematosis (SLE), mixed connective tissue disease, Sjogren syndrome, scleroderma (CREST) calcinosis, Rayaunds phenomenon, esophageal dysfunctions, sclerodactly and telangiectasia syndrom, rheumatoid arthritis and polymyositis.[3] The aim of this work is to studyANA,RF,&CRP and their titers in patients of SLE & RA in correlation with age and sex .

Materials and Methods

Patients: A total number of 103 patients with rheumatoid symptoms were included. The patients were admitted to Central Health Laboratories and Al-Yarmouk Hospitals from 2002 to 2003. Subjects included in this study were divided into following groups presented in table 1 .

Table (1) : number ,sex ,age and diagnosis of patients with SLE and RA

Disease	no	Age range	No of female	Age range	No. of male	Age range	Diagnosis
SLE	36	10-39	28	10-39	8	20-34	Depend on recognition of specific symptoms and identification of autoantibodies some of the most common symptoms butterfly (malar) rash,photosensitivity ,hemolytic anaemia [3]
RA	67	20-54	44	20-54	23	20-49	The typicalclinicalphenotype of is asymmetrical ,deforming , small and large jointspolyarthritis of ten associated with systematic disturbance and extrarticular disease feature .the clinical course is life long , with intermittent exacerbation and remission .[3]

All patients were referred to Immunological Laboratory of the two medical centers.

Blood Collection and manipulation : All samples were clotted at room temperature, centrifuged and the sera stored at –20 ^{0}C until used in the assays.

Rheumatoid Factor Test: - RF was assayed by the latex test Linear Chemicals, rapid agglutination, the determination is made by agglutination of the latex suspension with human gamma globulins in front of RF that found in the serum samples .The titer of

the serum is the reciprocal of the highest dilution that exhibits positive reaction Table (2).

Table (2):Dilutions of sera and the corresponding titers of ANA, RF and CRP

Dilution	Titer
1/1	1:2
½	1:4
¼	1:8
1/8	1:16
1/16	1:32
1/32	1:64
1/64	1:128

C-Reactive Protein Test: -is a rapid slide agglutination procedure (Linear Chemicals) based on a modification of the latex fixation method, developed for the detection and semi-quantization of C-RP in serum. The assay is performed by testing a suspension of latex particles coated with antihuman CRP antibodies against unknown serum. The presence of the CRP level above the upper limit of the reference interval in the sample was tested .

Antinuclear Antibody Test : - The SLE test is intended to be used as an aid in the diagnosis of Systemic Lupus Erythematosis through the detection and quantization of serum antinucleoprotein factors associated with SLE . One of the laboratory method to detect antinuclear antibody is agglutination of coated particles . The antibodies that are believed to be most characteristic of SLE are these that are directed against deoxyribonucleoprotein (DNP). The principle of this test is based on the agglutination reaction between latex particles coated with DNP (TDS company) being brought into contact with a serum, which contains antinuclear antibody. Agglutination indicates a positive reaction. The reaction time for this occurrence is within one minute.

Results

In table 3 and 4 the numbers and percentages of female and male patients positive (+ve) for ANA, RF and CRP are presented respectively togather with ages. The female: male ratios in SLE patients was 4:1 while for RA was 2:1. The peak age incidence of SLE was 20-24 years ,while that for RA 30-34 years . In male group there was no patients with SLE the following groups 10-14, 15-19 and 50-54, the incidence of SLE in the age range 10-19 was only in females. The peak age incidence of SLE and RA was 35-39 the percent was 27.8% of total number of female patients. The peak age incidence of male with SLE and RA was 25-29 and 30-34 respectively the percent was 32.3% of total number of male patients. In age groups (table 3) 10-14 and 15-19 all patients had a (+ve ANA), (+ve RF) and (+ve CRP). All patients with SLE had a (+ve ANA), (+RF) and (+ve CRP). While in RA patients not all patients had a +RF had +ve CRP except the age group 20-24. Two pf 35-39 had –ve RF but +ve CRP but they diagnosed as RA patients according to clinical manifestation. In 40-44 only one of RA patients had +CRP, while in age groups 45-49 and 50-54, no patients had +ve CRP. ALL patients with SLE had +ve RF and +ve CRP but not all patients with RA had +ve CRP.In table (5), 3 of 9 in age group 20-24 were makes 2 of them had titer 1:l6 and one had titer li8 of ANA, in age group 25-29 2 males had titer 1:2 in age group 30-34 three males had 1:2,1:4 and 1:8. In table (6), five males 20-24 three of them had 1:2 one had 1:4 another one had 1:l6. 10 males in 25-29 five with 1:2, 2 of them with 1:4 the other 2 with

1:8 and only one had 1:16. 10 males in 30-34, fifty percent had 1:64 and fifty percent had 1:128. In table 5, 6, 7 the only one female patient in age group 10-14 had the highest titer 1:128 in all parameters ANA, RF and CRP from these tables. While the female patients in age group 15-19 had the highest titers 1:32, 1:64 and 1:128 in ANA and RF but 2 patients had median titers in CRP 1:8 and 1:16, one had 1:32. In table (5) three females 33.3% in age group 20-24 had 1:32 titer and 60% had the highest titer 1:128.In table (5), 50% of patients in 25-29 were female and they had 1:160 the highest titer in this group. While in 30-34 the highest titer 1:8 was of one male. In table (6), the female patients in groups 10-14 and 15-19 had the highest titer of RF. In age group 20-24 female patients with SLE had the highest titers of RF (1:32), (1:64) and (1:128), while the 3 male patients with SLE had the titers of 1:2, 1:4 and 1:8, there were 2 females and 2 males with RA. Female with RA and SEL in age group (25-29) had the highest titers 1:32, 1:64, 1:128.

Discussion

SLE is the most common multisystem connective tissue disease. It is characterized by a wide variety of clinical features and diverse spectrum of autoantibody production. RA is the most common inflammatory and has an important case of potentially preventable disability. Many of the clinical features and management strategies in RA are relevant across the spectrum of inflammatory disease [4]. In our study 65% of patients were with RA while 35% of patients were with SLE, our RA percent similar to Julius et.al [9]. In this study all patients with +ANA were with SLE while in others ANA have been detected in less than 10% to greater than 70% of patients with RA [10,11], they found that 7.5% of RA patients were +ANA and RF –ve. The peak age incidence in the recent study was differ from that others, in the recent were (20-24) of SLE and (30-34) of RA, while the west last studies found that the peak age incidence of SLE (15-25) and (25-35) of RA. The incidence of SLE in female to male (4:1) and of RA (2:1) and this is differs from the last studies where the incidence of SLE in female to male (9:1) and RA (3:1). An increasing number of auto antibodies can be detected. Production of some of these is common, age related phenomenon that may be exaggerated by chronic inflammation. Their were presence, therefore, often has low diagnostic specificity and little clinical relevance. If present in high concentrations, however, their disease specificity often increases. It is therefore important to know how much antibody is present (the titer or concentration in unit) rather than just whether it is detectable [3]. The results show that the highest titers of some rheumatic diseases parameters (ANA, RF and CRP) were in females, this might due to the more sensitive immune response in female than male, in most non-rheumatic conditions, titers of RF are lower than in RA. [12]. The specificity of the RF reaction for RA increases with serum titers [13], and this differs form what was found in the recent study, because we found that the highest titers of RF was in the patients with SLE. (At least 50 auto antigenic targets for the auto antibody production described in the SLE . However , none of the diverse manifestations of SLE can be attributed to single antigenic stimulus, and likely that this wide spectrum of auto antibody production result from polyclonal. Many auto antigens in SLE are components of intracellular and intranuclear machinery

. B and T cell activation . In normal health these antigens are hidden from the immune system and do not provoke an immune response . Although the triggers that lead to auto antibody production in SLE are unknown , one mechanism may be expression of novel antigens on the cell surface during apoptosis . This hypothesis is supported by the fact that environmental factors that associated with flares of lupus increase oxidative stress and subsequent apoptosis . Such factors include exposure to sun light and artificial UV light , pregnancy and infection [3] or may be due to some genetic factors, so further analysis in families have a history of RA and SLE may be helpful in predicting the detection of immure response and precise genetic susceptibility to RA and SLE, it will also be useful to include other gene markers like α_1 anti trypsine, which are thought to be associated with severe seropositive RA and SLE[13]From table (3) and (4) we can conclude that the incidence of SLE in female only in the following groups (10-14), (15-19) and (35-39). And no female or male patients with SLE in the following groups (40-44), (45-49) and (50-54). Some studies have shown that the prevalence of RF and other auto antibodies in general population tends to decline beyond the age of 70 to 80 years [6]. This decrease may be related to an increased mortality rate among autoautibody-positive individuals , but in Iraq because of short life expectancy in comparison to the western countries and because of inability of the elderly people to contact health system due to their low socioeconomic state . Further studies include HLA-D locus situated on chromosome 6 in man. Research on etiology of the diseases association with human leucocytes antigen drew attention to the possibility of genetic predisposition coded at or near the HLA-D locus immune response gene which may be in linkage disequilibria with HLA-D locus[14].

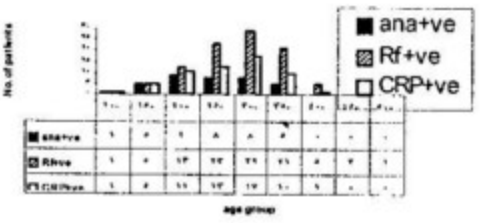


Figure (1): Age Distribution Pattern of patients According to ANA+,RF+, CRP+

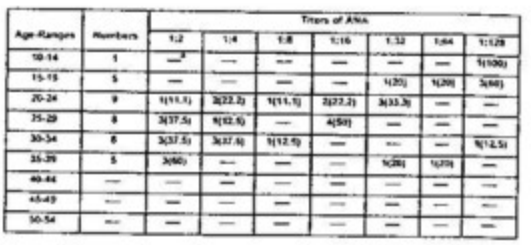
Table (3) : Numbers and Percents of female patients with ANA+ve, RF+ve and CRP+ve with age ranges

age ranges	Total No.%	ANA +ve no. %	RF+ve no.%	CRP +ve no.%
10-14	1(1.4)	1(100)	1(100)	1(100)
15-19	5(6.9)	5(100)	5(100)	5(100)
20-24	8(11.1)	6(75)	8(100)	8(100
25-29	13(18)	6(46.2)	13(100)	9(69.2)
30-34	19(26.4)	5(26.3)	19(100)	11(58)
35-39	20(27.8)	5(25)	18(90)	9(45)
40-44	3(4.2)	zero	3(100)	1(33.3)
45-49	2(2.8)	zero	2(100)	zero
50-54	1(1.4)	zero	1(100)	Zero

Table (4) : Numbers and Percents of male patients with ANA+ve, RF+ve and CRP+ve with age ranges

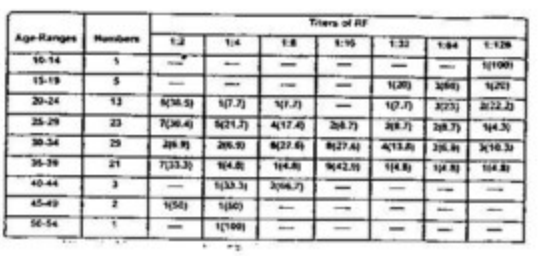
age ranges	Total No.%	ANA+ve no.%	RF+ve no.%	CRP+ve no.%
10-14	zero	zero	zero	zero
15-19	zero	zero	zero	zero
20-24	5(15.1)	3(60)	5(100)	3(60)
25-29	10(32.3)	2(20)	10(100)	5(50)
30-34	10(32.3)	3(30)	10(100)	5(50)
35-39	3(9.7)	zero	3(100)	1(33.3)
40-44	2(6.4)	zero	2(100)	zero
45-49	1(3.2)	zero	1(100)	zero
50-54	zero	zero	zero	zero

Table (5): Numbers and percents of female and male patients with titers of ANA for each age group



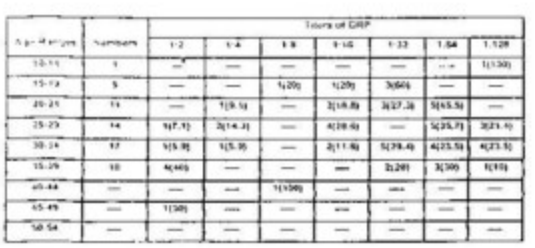
*** No patient have this ratio in the sample.**

Table (6): Numbers and percents of female and male patients with titers of RF for each age group.



***No patient have this ratio in the sample.**

Table (7): Numbers and percents of female and male patients with titers of CRP for each age group.



*** No patient have this ratio in the sample.**

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دراسة ANA , RF, CRP في مرضى مصابين ببعض الأعراض الروماتيزمية

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الخلاصة:-

تم قياس مستويات ANA,RF,CRP في مصول ١٠٣ مريض مصاب بأعراض روماتيزمية وكان ٣٦ مريض أي ٣٥% من المرضى مصابين بداء الذئب الأحمراري SLE و٦٧ أي ٦٥% من المرضى مصابين بالحمى الروماتيزمية ، وكانت الإصابة بداء SLE ١:٤ في الإناث عنهم في الرجال بينما الإصابة ب RAكانت ١:٢ ز إن أعلى مستوياتANA,RF,CRP كانت في النساء . كانت الإصابة بSLE بالنساء فقط في المديات العمرية الآتية (١٠-١٤) و (١٥-١٩) و (٣٥-٣٩) ، في حين لا توجد نساء ولا رجال مصابين ب SLE في المديات العمرية الآتية (٤٠-٤٤) و (٤٥-٤٩) و (٥٠-٥٤) .