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EVALUATION OF ACUTE PHASE PROTEINS AND ANTI-CYCLIC CITRULLINATED PEPTIDE (ANTI-CCP) LEVELS IN RHEUMATOID ARTHRITIS

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ABSTRACT : Rheumatoid arthritis (RA) considered autoimmune disease have an effect on various tissues, especially joints. To evade joint destruction, organ damage and disability; early diagnosis as well as treatment are the most suitable. The study the level of "anticyclic citrullinated peptide" (anti-CCP) in rheumatoid arthritis patients, in addation to study the relationship between the "anti- CCP" and the parameters of disease activity in "RA" patients were the aim of this study. Fifty-two "RA" patients compared with 40 controls were enrolled in this study. Sera were tested for "erythrocyte sedimentation rate" (ESR), "C-reactive protein" (CRP)," rheumatoid factor" (RF). "Anticyclic citrullinated peptide antibody" (anti-CCP) was determined by "Enzyme Linked Immunosorbent assay" (ELISA). High significant difference (P<0.01) in the concentration of anti-ccp antibodies, RF, ESR and CRP between RA and control group. A significant correlation betweenAnti-CCP antibody level and ESR, CRP (r = 0.59, P<0.01) among RA patients. But, no correlation between anti-CCP antibody and RF. Anti-ccp antibodies, rheumatoid factor, erythrocyte sedimentation rate and C-reactive protein may be the prognostication parameter of RA in the rheumatoid arthritis patients.

Key words : Anti-ccp antibodies, rheumatoid arthritis, RF, ELISA.

INTRODUCTION

"Rheumatic diseases" are considered health problems have an effect on people around the world, leading to destruction, deformities of joints and function disability (Scott and Tofacitinib, 2013; Haro and Sanmarti, 2013). RA affects approximately 1% of the world population, being more in women than men 2:1 to 3:1 (Viatte *et al*, 2013) in age between 40 and 60 years old, and it causes premature death (Vinary *et al*, 2013). Many studies have shown that the disease progresses rapidly during first two years of onset and can lead to irreversible erosive joint destruction (Combr, 2009). The mannerto avoiding joint destruction and organ damage required early diagnosis and treatment of "RA", particularly in the early course of disease (O´Dell, 2005). Genetic and environmental factors play important role in development of clinical aspects of the disease, like many other diseases (Berglin *et al*, 2004). Genetic factors are linked with a series of genes that carry information that related with RA, particularly the HLA-DRB1 accounting for about one-third of the genetic risk of RA (Fauci and Langford, 2013). A decisive diagnosis of "RA" basis on articular indication of the disease, radiographic results, immunological markers as well asmany of autoantibodies have recently been associated with disease activity and/or prognosis of "RA" (Kasper *et al*, 2005). The RF is detected in approximately 50-80% of patients with RA. Elevated serum level of RF has been related to higher disease activity, radiographic progression, bone erosion and disease outcome (Matuszewkam *et al*, 2015). Rheumatoid factor remains one of the ACR classification criteria for RA, its diagnostic value is unsatisfactory, especially in early disease because other diseases such as"Sjogren syndrome", "systemic lupus erythematosus", "leprosy", "hepatitis B", "tuberculosis" even(5%) of healthy people have positive rheumatic factor. Over two decades, conclude that highest specificity of "RA" are autoantibodies that directed against proteins containing "citrulline epitopes". These antibodies are called "ACPA" which first described as "anti-perinuclear factor antibodies" (APF) (Nienhuis *et al*, 1964). The anti-CCP antibodies are mainly of the IgG class, although IgM and IgA anti-CCP can also be detected (Rantapaa-Dahlqvist *et al*, 2003). Young *et al* (1979) was seen the sera of ''RA" include antibodies that reacted to "layer of keratinized" of epithelial cell; named" antikeratin antibodies", which only present in "RA" patients and suggest they have a role in pathogenesis of RA (van Venrooij and Pruijn, 2008). Many studies have pointed

out that "anticycliccitrullinated peptide antibody" (anti-CCP) titers can be benefit in diagnosing of ''RA and considered as prognostic indicatorfor joints destruction in "RA". Obviously that detecting "RA" at beginning of its stages isimportant for treatment and to limit its complications (Irandokht *et al*, 2016). Acute phase reactants such as "C reactive protein" (CRP) is possible for increased risk of "RA" and disease activity its concentration increases in the blood after various stimuli. CRP is a part of ACR core data set for measuring disease activity in RA. In contrast the mostmarkers used to measure disease activity are "ESR" and its remains the best tool for detecting inflammatory diseases and "RA" (Combe *et al*, 2001; Wolfe *et al*, 2001). In RA, ESR has been shown to correlate with outcome and to influence radiological progression in many studies. As a consequence, the "ACR" and "European League Against Rheumatism" (EULAR, 2010) has improve criteria to classify "RA" based on new parameters such as "anti-citrullinated peptide antibodies" (anti- ACPA) can be used as new diagnostic tools for "RA" in an earlier phase of RA (Aletaha *et al*, 2010). C-reactive protein (CRP), erythrocyte sedimentation rate (ESR) and "Rheumatoid factor" (RF), these criteria stating to identify "RA" patients early in order to precede drug therapy and to minimize the functional disability. In the present study, we have investigated the correlation of the presence of anti-CCP antibody with disease activity.

MATERIALS AND METHODS

The study consisted of 52 RA patients (42 females and 10 males) take part in this study. The diagnosis of those patients has been performed under supervision of a specialist physician in rheumatology department in Baghdad Teaching Hospital, Iraq. The age group for RA patients were range from (<40; 40-50; <50) with average (30.09; 46.23; 56) respectively. 5 ml of blood was collected from both groups, subjected to centrifugation to get serum and stored at 4ºC.

Serum "RF" concentration was determined by immunonephelometry method on BNII nephelometer (Dade Behring, Marburg, Germany). RF concentration higher than 15 IU/ml were considered positive. ELISA was used to determine level of serum CCP of both groups employ a commercially available "Human anti-CCP ELISA kit" (Euroimmun-Germany). These assays were implemented according to the manufactures (cut off value, 5 IU/ml). "Avitex–CRP" kit is fast "latex agglutination" test was used for detection of CRP in the serum. Its principlebased on that "Avitex- CRP latex" particles are covered with antibodies belongs to human CRP. If serum concentration of CRP is more than 6mg/liter means positive. Erythrocyte sedimentation rate "ESR" is detected by "Westergreen sedimentation-rate testing". Whereas the positive value of ESR test per hour is more than 15 and 20 mm for male and female, respectively.

Statistical analysis

The system of statistical analysis used to study parameters was (2012). Thecomparisons between means were analysis by using T-test or LSD test. A correlation coefficient between variables has been carried out in this study.

RESULTS

Table 1 shows the highest percentage of patients 21 (40.3%) is among more than 50 years. While the age group less than 40 years was 18 (34.6%). Here as the lowest percentage 13 (25%) among the study sample is 40-50 years. As well as the table shows the highest percentage of patient was women 42 (80.71%), while men were 10 (19.2%).

Table 2 shows that highly significant differences (P value< 0.01) between RA patient and control group in levels of ESR (58.76 and 13.75), CRP (27.38 and 3.87), RF (109.57 and 5.18) and Anti-CCP (101.08 and 4.04), respectively.

Table 3 shows significant correlation between "Anti-CCP" and disease activity parameters (ESR and CRP) (r=0.59, r=0.59), respectively.

Table 4 shows no significant correlation between "Anti-CCP" level and rheumatoid factor ($r = 0.13$, $p > 0.05$).

DISCUSSION

RA affects approximately 0.5-1% of the world population (Hambright *et al*, 2011) being more common in women than in men (Ebringer *et al*, 2010). In the present study, male to female ratio was1: 4.19 that correlates with the studies conducted by Bushra *et al* (2014), Malini *et al* (2017), Fazal *et al* (2017), Kiran *et al* (2018) were reported that women are more commonly in risk of RA and are affected 2 to 3 times more than men with ratio 1:4. Further studies by Pedersen *et al* (2007), Yadollah *et al* (2014), Ihsan *et al* (2015), Bineeta *et al* (2015), Mohammad *et al* (2017) conducted that women are more affected with "RA" than men with ratio of 3 to 1 and that may be due to estrogen hormonal that inhibits the "T-suppressor" cells functions and enhances "T-helper" cells; addition to thatreceptors of estrogen are present on cells of synovial (Nalbandian and Kovast, 2005).

The ultimate risk factor for "RA" is aging due to diminish of normal immune surveillance also dysregulation (Goronzy and Weyand, 2007). ''RA" incidence move

Table 1 : Age and sex distribution of Rheumatoid arthritis (n=52).

Age (yrs)	<40	40-50	>50	Total
Average (yrs)	30.09	46.23	56	
Total	18 (34.6%)	13 (25%)	21 (40.3%)	
Sex	Female	42 (80.71%)		52 (100%)
	Male	10 (19.2%)		

Table 2 : Concentration of ESR, CRP, RF and Anti-CCP in "RA" and control group.

Parameters	RA patients (n=52)	Control (n=40)	P-value
ESR (mm/hr)	58.76±5.22	13.75±1.22	p<0.01
CRP (mg/dl)	27.38±4.91	3.87±0.49	p<0.01
RF (IU/ml)	109.57±24.12	5.18±0.89	p<0.01
Anti-CCP (U/ml)	101.08±14.24	4.04±0.71	p<0.01

Table 3 : Correlation between Anti-CCP and CRP level and ESR.

Parameters	Correlation coefficient-r	Level of sig.
ESR	0.59	p<0.01
CRP	0.59	p<0.01

Table 4 : Correlation between rheumatoid factor and Anti-CCP in "RA".

Parameters	Correlation coefficient-r	Level of sig.
RF+ Anti-CCP	0.13	P>0.05

upwards dramatically throughout ''adulthood" extend to its high pointat aged 40 to 60 years (Doran *et al*, 2002). In the present study, the majority of ''RA" patients observed in the age group above 50 years. The results confirmed by Alamanosa and Drosos (2005), Abdullah (2010), Jasim (2012), Doðan *et al* (2014), Maria *et al* (2014), Ihsan *et al* (2015), Fazal *et al* (2017). Current research suggests that immunosenescence that occurs with aging can lead to chronic inflammation and immune-mediated tissue damage (Weyand and Coronzy, 2014).

The criteria of "ACR/EULAR RA classification" in 2010 was included; ''anti-CCP", RF and parameters of disease activity for early diagnosis and prevention of irreversible deformities, that included; small joint involvement, disease duration, positive results for rheumatoid factor (RF) or anti-cyclic citrullinated peptide (anti-CCP) and presence of any one of the acute phase inflammatory markers (Aletaha *et al*, 2010).

The present study found that serum level of "anti-CCP", RF and disease activity "ESR , CRP" statistically "highly significant" increase in between "RA" and control groups (p<0.01). The studies by Hanan *et al* (2011), Merza *et al* (2014), Ihsan *et al* (2015), Rongchun *et al* (2015), Yousf anbd Khalid (2015), Irandokht *et al* (2016), Mohammad *et al* (2017) all support the study that levels of serum "CRP, ESR, Anti-CCP levels and rheumatoid factor" in patients with "rheumatoid arthritis" were statistically significantly higher than control group. The characteristic features of "rheumatologic diseases" are inflammatory responses which denote to damage of tissue get rid of "pathogens", restrict injury addition to that tissue regeneration will occurs. These changes collectively as a result either increases or decreases of some "certain proteins". The levels of these "acute phase proteins" with clinical information are used to evaluate "disease activity" and response to treatment (Murat *et al*, 2017).

Therefore to confirmed diagnosis of "RA" are by the detection of "RF, anti-CCP antibody, CRP and ESR". In this study, we found significantly correlation of level of anti-CCP antibodies with erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP). Similarly studies by Doðan *et al* (2014), Arbi *et al* (2015), Bineeta *et al* (2015), Ihsan *et al* (2015), Mohammad *et al* (2017) they found significantly correlation between "anti-CCP antibodies" and higher activity marker "ESR, CRP". Laboratory markers "RF and anti-CCP antibody" are sensitive for the diagnosis of "RA". Studies by Hanan *et al* (2011), Doðan *et al* (2014), Banseok *et al* (2018) found that "anti- CCP antibody" and rheumatic factor was correlation significantly. Otherwise, we find no correlation between "anti- CCP antibody and RF" and that confirmed by Suzuki *et al* (2017). The current study had a number of limitations that may effect on the results; the limited number of enrolled patients, the lack of therapeutic information on arthritis. Association of "anti-CCP" with disease activity in RA proposes that "anti-CCP" is more beneficial than only RF in the diagnosis and distinguishing "RA" from other inflammatory diseases.

CONCLUSION

Anti-CCP assay could be early marker of rheumatoid arthritis as predict the severity of the disease addition to that could help in prevention joint damage. The use of "Anti-CCP" in clinical practice jointly with RF, could participate in treatment decision.

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