Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 408 - 418
Received 05 March 2021; Accepted 01 April 2021.

The Correlation between ACCP with Developing, Progression and Activity of Rheumatoid Arthritis

InamTahseen Alwan[1*], Kareem Hamed Ghali[2]
[1, 2] University of Wasit, College of Science, Department of Biology, Iraq
*Corresponding author: enamt81@gmail.com

ABSTRACT

Rheumatoid arthritis (RA) is a common autoimmune disease and is characterized by chronic inflammation that affects the joints and leads to joints damage and losing their normal functions and chronic pain. Rheumatoid arthritis disease is prevalent worldwide and affects both sexes and all ages. The current study included seventy-five patients positive for rheumatoid factor attending the hospitals and private clinics.This study aims to investigation the correlation between ACCP and development, progression, and activity of RA in Iraqi patients. ACCP was analyzed using ELISA technique,using Sandwich-ELISA method. The results of the study showed that the percentage of women patients is 88% and the rest are males. The most affected age group is between 32-41 years, and its rate is 29%. As well as the results showed the levels of ACCP in rheumatic patients were high than in control group with significant increase in patients ($p<0.001$). The results also showed that ACCP increased with the progression of disease activity, reaching the highest level of this factor in sever status ($p<0.02$).Our results conclude the increase of ACCP in serum of RA patients was associated with development and progressionof rheumatoid arthritis in Iraqi patients, while there is no effect of sex, age, or associated diseases on the level of ACCP in patients serum.
Keywords: Rheumatoid arthritis, ACCP, diabetes, hypertension, autoimmune disease

Introduction

Rheumatoid arthritis (RA) is chronic autoimmune disease, causes progressive articular damage, functional loss, and comorbidity [1].RA disease affects both sexes, but it is more common in females. Rheumatoid arthritis is a systemic disease that affects various organs of the body such as the lung, heart, eyes, hands, and red blood cells [2] . The incidence of this disease is relatively stable and ranges from 0.5 to 1% with some exceptions [3].Although the causes of the RA disease are not known precisely until now, genetic and immunological causes have main role in developing the disease in addition to the severity and progress of the disease.Rheumatoid arthritis is related with high incidence of mortality, and fifty percent of patients have risk of premature mortality [4]. Citrulline is a noncoding native amino acid present in two forms,free amino acid and a peptidyl form. Anti-cyclic citrullinated peptide (ACCP)recognize citrulline [5].The anti-Cyclic Citrullinated Peptide Antibodies are produced locally in the inflamed synovium RA patients [6]. Over the past 20 years, ACCP antibodies are used as predictive, diagnostic differential diagnostic tool and treatment monitoring for RA [7].The elevated ACCP level was observed with a number of pathological aspects associated with AR such as disease severity and disease activity, more severe, joint damage, worse functional disability and reduced quality of life [8,9].This study tries to finding the correlation between ACCP levels with the development, progression, andRA activity in Iraq patients.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 408 - 418
Received 05 March 2021; Accepted 01 April 2021.

Materials and Methods

Seventy five patients with positive rheumatoid factor (RF) and eight healthy individuals as control group are included in this study. The patients are attended to the Rehabilitation Center for the Disabled and Artificial Limbs and Al Zahra'a and Al Karama Teaching Hospitals in Wasit Province from 1 December 2019 to 1 December 2020. The ages of the patients ranged from twenty two to ninety-three years, and the severity of the disease was classified from the patients. In addition the laboratory test Erythrocyte Sedimentation Rate (E.S.R) was checked for each patient .The clinical data from patients were recorded such as sex, disease severity, disease duration, line of treatment and associated diseases . The healthy individuals had no pathological state at time of this study, and without any history of systemic diseases, immunological disorders, and cancer diseases. Only two ml of blood were collected from all patients positive for rheumatoid factor (RF) and control group, then the serum was separated in jell tube.

ACCP analysis

ACCP was analyzed usingSandwich- ELISA kitas described by the manufacturer (Elabscience Biotechnology Inc.) . The micro ELISA plate is covered by antigen specific to human ACCP antibody. The results were read by ELIZA reader at 450 nm wavelength.

Statistical analysis

Patients data were tabulated and processed using SPSS (Statistical package for the social sciences) V.20. Quantitative data were calculated as mean, SE and SD. The difference between means assessed by analysis of variance (one and two way -ANOVA). P-value for all tests was considered significant if <0.05.

Results and Discussion

Distribution of RA patients according to sex and age group

Sex and age group of Rheumatoid Arthritis patients were summarized in table(1) of 75 patients , and were divided to 9 males(12%) and 66 females (88%) with P value < 0.05.While age group were ranged between 22 year to up 62 year, with mean (52.5)years and P value >0.05. Distribution of cases according to sex group showed that the RA disease was found in females more than males .These results were in agreement with many previous researches , Couderc. *et al* ., (2014)[10] showed it is clearly established that RA affects women more frequently than men, with a gender ratio of 3:1 . It has been suggested that sex hormones are the reason for the difference, based on the inductive role of females. This study also grouped RA patients into five age groups as shown in table(1),and we observed that the most effected group was in (32-41years) age group with percent 29,3% and this result is coming in agreement with the result of Bajocchi*et al* ., (2000) .It has been reported that RA in men increases slightly with age, and in women it increases from 45 years of age [11] .

Generally, as mentioned in the above studies the RA disease was seen in females more than males and the increase of RA associated with advance of age and the incidence of disease after 55 years of age. Moreover, Vollenhoven (2009)[12] showed the sex differ in functional capacity for patients with RA . The interesting opinion reported by Vollenhoven (2009)[12] indicated that X-linked genetic factors (X-linked is a trait where a gene is located on the X chromosome), hormones and differences in exposure routes between women and men may influence the incidence and severity of autoimmune diseases. In the same direction ,Kvien. *et al*., (2014)[13] indicated that the number of rheumatoid arthritis females four to five times more than males

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 408 - 418
Received 05 March 2021; Accepted 01 April 2021.

below the age of 50 years. Moreover, the results showed that the number of patients who live in urban places is higher than that in rural places.AS well as.Collectively previously studies established this fact andwe also confirmed this result.

Assessment the Levels of ACCP in RA Patients and Control Group

The levels of ACCP in rheumatic patients and control group were summarized in table(2), the mean level of ACCP was recorded , in patients as 196.6 IU/ml and in control group 95.7 IU/ml with significantly increase in patients than in the control group ($p < 0.001$) . Our results indicated ACCP level increasedin RA patients compare to control group. These results were coming in agreement with many researches [14]. Chou. *et al* ., (2007)[14] study a total of 126 cases and they observed that ACCP level was significantly increase in RA patients compared with control group ($p < 0.01$).Therefore ACCP is a good clinical marker for RA diagnosis and detection the RA severity. The study of 150 patients with longstanding RA showed that Anti-CCP has high specificity and moderate sensitivity in the early presentation of RA patients with significantly increased in patients compared to the control group ($p < 0.01$) [15]. Generally ,as mentioned in the above studies the level of Anti-CCP has become a key serologic marker in RA disease . It can be“ used (1) as a test for early diagnosis of RA; (2) for the differential diagnosis between RA and other rheumatic or immune diseases; (3)for prediction of prognosis; and (4) for evaluation of treatment outcome”) [14]. ACCP is an important seriological marker for RA diagnosis due to its high specificity for RA ,and the use of ACCP with RF increases the possibility of RA diagnosing at an early stage [16].

As well as ,Alfatlawi. *et al* ., (2020)[16] found in their study, a strong positive association between ACCP log 10 and RF log 10 for males and females with RA disease. We concluded ACCP is a good indicator for RA diagnosis as compared with rheumatoid factor (RF). A lot of studies have impeached the magnitude of ACCP testing in differentiation RA from other of inflammatory-diseases. Although the role of ACCP in AR patients is still unclear, it may be associated with increased susceptibility to this disease in healthy subjects, and it can be diagnosed in the blood of healthy individuals prior to clinical RA [17] .Our study showed an association between the increase of ACCP level and rheumatoid arthritis development in Iraqi patients .

Assessment the level of ACCP titer in RA patients according to sex

Table (3) showed the level of ACCP in rheumatoid patients according to sex, the results showed the mean level of ACCP in female rheumatoid patients was (196.6±(7.6) ±(9.4)) while in male rheumatoid patients was (196.4 ±(6.2) ±(2.0)), with no significant difference ($p =0.9$) .These results were in agreement with many obvious papers [18]. The results of this study indicate that sex may not effect on ACCP level in RA patients. However , in another study performed by Shafiaa*et al* ., (2016)[19], showed a significant association between ACCP level and the female gender ($p< 0.01$).

Assessment the level of ACCP titer in RA patients according to age groups

Table (4) showed the level of ACCP in RA patients according to age groups .According to age groups of patients, the results showed that the first group of patients (22-31 Y) recorded 181.8(4.8)±(1.7) IU/ml , while the second group (32-41 Y) , 207(7.7)±(1.6) IU/ml ,the third group (42-51 Y) , 167.9(7.6)±(1.9)IU/ml ,the fourth group (52-61 Y) , 192.7(7.6)±(1.9)IU/ml , and the last group (61+ Y) recorded 215(8.3)±(2) IU/ml with no significant differences ($p> 0.05$) between the five age groups regarding ACCP levels .Our results indicates to no effects of age on

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 408 - 418
Received 05 March 2021; Accepted 01 April 2021.

ACCP concentration in RA patients serum . Our results were coming in agreement with many researches [20,21] .However , Alfatlawi. *et al* ., (2020)[16] showed that ACCP levels were increased with the age in RA male and female patients with P-value < 0.001, 0.004 respectively. CT Chou. *et al* ., (2007)[14] study 155 rheumatism patients divided into ACCP-positive and ACCP-negative groups, and they did not find a significant difference between ACCP values based on age, gender, disease period and treatment. Pawłowska. *et al* ., (2011)[22] suggest the chances of the RA disease advance with age and onset of disease at 40 years .

Assessment the level of ACCP titer in RA patients according to associated diseases

Distribution of ACCP Levels in rheumatoid patients according to associated diseases was shown in table (5). The mean level of ACCPP in rheumatoid arthritis patients with diabetic disease was(213.5 IU/ml), with hypertension (145.1 IU/ml),with other disease (158.3 IU/ml) and rheumatoid arthritis patients only was(204.4 IU/ml).The results showed no significant difference between all groups regarding the concentration mean of ACCP in patients . Our study agrees with the study of Twigg*et al* ., *(2018*)[23] , who reported that there was no significant difference in CCP antibodies among RA cases with other associated diseases .Also , Barbarroja. *et al* ., (2014)[24] study a total of seventy-five RA patients and 31 cases as control group and they showed no significant correlation between the traditional risk factors (obesity, diabetes mellitus, and hypertension) and the inflammatory parameters and oxidative markers analyzed in RA patients . In the same line , LO´ PEZ-Longo *et al* ., (2009)[25] showed ACCP levels in RA patients were not associated with the development of ischemic heart disease and diabetes mellitus.

Assessment the level of ACCP titer in RA patients based on the severity of the disease

Table (6) showed the distribution of ACCP level according to severity of RH disease in Iraqi patients ,the mean levels of ACCP were highly increased in the sever and moderate disease (206.4 U/ml)when compared with non sever (Low-new-inactive) (145.1 U/ml) with significantly difference (P value $\leq$ 0.01). The levels of ACCP can be used in differentiation between the three degree of RA activity .In addition, Sanmarti*et al* ., (2007)[26] and Fathi*et al* ., (2008)[27] reported that the higher levels of ACCP or RF was refer to high grade of RA activity. Our results concluded that ACCP level in serum of RA patients may be used as an important indicator for aggressive disease .

Assessment the level of ACCP titer in RA patients according to activity of disease

In this study, RA patients demonstrated different levels of ACCP based on disease activity (table 7) .The relationship between ACCP titer according to the activity of disease in rheumatic patients was showed in the table (7).The results showed the distribution of ACCP levels in three categories of disease activity , the low activity was recorded 145.1 ±(5.1) ±(1.4) IU/ml , moderate activity was 203.6 ±(8.3) ±(1.4)IU/ml ,and sever activity was 209.2±(6.5) ±(1.1) IU/ml . The results showed a gradually increase in the level of ACCP in RA patients with the progression of the activity with a significant difference (P value≤0.02). Measuring the level of ACCP in RA patients is very important because it may indicate broken joints and bone loss [28] . The ACCP level was increased with higher DAS28 score also showed in Moroccan RA patients [28] . The current results were in agreement with many previous studies [20,29].However, some studies carried out in different regions indicated no relationship between ACCP level and rheumatic disease activity [2,21,30,31].

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 408 - 418
Received 05 March 2021; Accepted 01 April 2021.

Assessment the level of ACCP titer in RA patients according to t line of treatment

Relationship between ACCP titer and treatment of disease in rheumatic patients were summarized in table(8) .Out of 75 rheumatoid patients , seven patients were treated with vitamins and showed 155(5.1)± (1.9) ,IU/ml ACCP , 10 patients treated with Painkiller 197.5(8.3)± (2.6), IU/ml ACCP ,44 patients treated with Chemotherapy,197.1(6.9)± (10.5)IU/ml and14 patients of them were treated with Steroid ,214.9(8.9)± (2.4)IU/ml. The level of ACCP showed no significant difference according to the type of treatment. The results showed that the medications used had no effect on ACCP levels, which means that these drugs did not modulation or alter ACCP production in rheumatic patients. These results are consistent with other studies, Barbarroja. *et al* ., (2014)[24], study a total of seventy-five RA patients treated with corticosteroids ,Anti-malarials, ,NSAIDS, Metotrexate, Anti-TNF treatment and Vitamin D. Other study showed the most common treatments involved a mixture of medicines[31]

Table 1. Distribution of RA patients according to sex and age group

RA patients	**No %**	**P value**
Males	9 (12%)	0.9
Females	66 (88%)	
Total	75 (100%)	
Age range	22-83	
Age mean (year)	52.5	
Distribution of patients according to age groups		
22-31 Y	8 (10.67%)	0.44
32-41 Y	22 (29.3%)	
42-51 Y	13 (17.3%)	
52-61 Y	15 (20%)	
62+	17 (22.67%)	
Total	75	

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 408 - 418
Received 05 March 2021; Accepted 01 April 2021.

Table 2. Levels of ACCP in RA patients and control group

Cases	No.	Mean of concentration mean SD±SE±IU/ml	P value
Rheumatoid arthritis patients	75	196.6 ± (7.4), ±(8.5)	0.001
Control group	8	95.7± (9.2), ±(3.2)	

Table 3. Distribution the level of ACCP titer in RA patients according to sex

Cases	No. %	Mean of concentration SD±SE± IU/ml	P value
Male rheumatoid patients	9 (12)	196.4 ±(6.2) ±(2.0)	0.9
Female rheumatoid patients	66 (88)	196.6±(7.6) ±(9.4)	
Total	75		

Table 4. Distribution the level of ACCP titer in RA patients according to age groups

Age of patients	No. %	ACCP concentration Mean SD±SE±
22-31 Y	8 (10.67)	181.8(4.8)±(1.7)
32-41 Y	22 (29.33)	207(7.7)±(1.6)
42-51 Y	13 (17.33)	167.9(7.6)±(1.9)

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 408 - 418
Received 05 March 2021; Accepted 01 April 2021.

52-61 Y	15 (20)	192.7(7.6)±(1.9)
62+ Y	17 (22.67)	215(8.3)±(2)
P value		0.44
Total	75	

Table 5. Distribution of ACCP Levels in RA patients according to associated diseases

Type of associated diseases	**No. of patients**	**ACCP mean concentration SD± SE±IU/ml**
Rheumatoid arthritis patients with diabetes	12	213.5±(8.2)±(2.3)
Rheumatoid arthritis patients with hypertension	7	145.1±(3.5)±(1.3)
Rheumatoid arthritis patients only	50	204.3±(7.2)±(1)
Rheumatoid arthritis patients with Other disease	6	158.3±(8.3)±(3.4)
P value		0.1
Total	75	

Table 6. Distribution of ACCP Levels in RA patients according to severity of diseases

Severity of disease	**patients No. %**	**ACCP mean concentration SD± SE±IU/ml**	**P value**
Sever(sever -moderate)	63 (84)	206.4 ± (7.4), ± (9.3)	0.01
Non sever(low-new - inactive)	12 (16)	145.1± (5.1), ± (1.4)	
Total	75		

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 408 - 418
Received 05 March 2021; Accepted 01 April 2021.

Table 7. Distribution of ACCP Levels in RA patients according to RA disease activity

Activity of disease	**No. of patients**	**ACCP mean concentration SD±SE± IU/ml**	**P value**
Low	12	145.1 ±(5.1) ±(1.4)	0.02
Moderate	32	203.6 ±(8.3) ±(1.4)	
Sever	31	209.2±(6.5) ±(1.1)	

Table 8. Distribution of ACCP Levels in RA patients according to treatment line

Type of treatment	**Patients No. %**	**ACCP mean concentration SD±SE±IU/ml**	**P value**
Vitamins	7 (9.3)	155(5.1)± (1.9)	0.39
Painkiller	10 (13.3)	197.5(8.3)± (2.6)	
Chemotherapy	44 (58.7)	197.1(6.9)± (10.5)	
Steroid	14 (18.7)	214.9(8.9)± (2.4)	
Total	75		

Conclusion

Our results conclude the increase of ACCP in serum of RA patients was associated with development and progression of rheumatoid arthritis in Iraqi patients

Acknowledgement

Special thanks with respect to Dr.Kahtan Adnan Hafedh D.R.M.R Specialist of Rheumatology &Medical Rehbilitation in Al-karama Teaching Hospital ,Dr.JalalTuffah ,AqeelNaji and Ali Abd Al-Kadhum from Al kut Hospital and InassTahseen from Wasit Health Department for their unlimited help. We are grateful to Dr. Safaa Abdul Allah/College of Medicine, WasitUniversity, for helping us in the analysis of results.

References:

[1] Yang Yuanyuan ,Payal Deshpande, Karthik Krishna, VinodhRanganathan, VasanthJayaraman, Tianhao Wang, Kang Bei, John J. Rajasekaran, and Hari Krishnamurthy.(2019). Overlap of Characteristic Serological Antibodies in Rheumatoid Arthritis and Wheat-Related Disorders . Disease Markers . ID 4089178, 6 pages https://doi.org/10.1155/2019/4089178

[2] Sulaiman F., N.; Wong K., K.; Ahmad W., A.,W. and Ghazali W., S., W. (2019).Anti-cyclic citrullinated peptide antibody is highly associated with rheumatoid factor and radiological defects in rheumatoid arthritis patients . Medicine 98:12(e14945):1-6

[3] Silman Alan J and Pearson Jacqueline E .(2002). Epidemiology and genetics of rheumatoid arthritis. Arthritis Research Vol 4 Suppl 3, S265-S272

[4] MyasoedovaE. , John M. Davis III , Cynthia S. Crowson&Sherine E. G.(2010). Epidemiology of Rheumatoid Arthritis: Rheumatoid Arthritis and Mortality . . CurrRheumatol Rep, 12:379–385,DOI 10.1007/s11926-010-0117-y

[5] Yamada R., Suzuki A.& Yamamoto K.(2006). Citrulline and anti-cyclic citrullinated peptide antibodies in rheumatoid arthritis . Future Rheumatol. 1(2): 249–258

[6] Ballini A., Tetè S., Scattarella A., Cantore S., Mastrangelo F., Papa F., Nardi GM., Perillo L., Crincoli V., Gherlone E., Grassi F R.(2010). The role of anti-cyclic citrullinated peptide antibody in periodontal disease. Int J ImmunopatholPharmacol . 23(2):677-81. doi: 10.1177/039463201002300234.

[7] Taylor P., Gartemann J., Hsieh J., and Creeden C. (2011). A Systematic Review of Serum Biomarkers Anti-Cyclic Citrullinated Peptide and Rheumatoid Factor as Tests for Rheumatoid Arthritis. Autoimmune Diseases ,Volume 2011, Article ID 815038, 18 pages doi:10.4061/2011/815038

[8] Mjaavatten MD, van der Heijde D, Uhlig T, Haugen AJ, NygaardH,Sidenvall G (2010). The likelihood of persistent arthritis increases with the level of anti-citrullinated peptide antibody and immunoglobulin M rheumatoid factor: a longitudinal study of 376 patients with very early undifferentiated arthritis.Arthritis Res Ther; 12: R76.

[9] Ibn Yacoub Y, Amine B, Laatiris A, Hajjaj-HassouniN.(2012). Rheumatoid factor and antibodies against citrullinated peptides in Moroccan patients with rheumatoid arthritis: association with disease parameters and quality of life. ClinRheumatol; 31: 329–334.

[10] Couderc M., Gottenberg J., Mariette X., Pereira B., Bardin T., Cantagrel A., Combe B., Dougados M., Flipo R., Le Loët X., Shaeverbeke T., Ravaud P., andSoubrier M..(2014). Influence of gender on response to rituximab in patients with rheumatoid arthritis: results from the Autoimmunity and Rituximab registry. Rheumatology, 53(10): 1788–1793, https://doi.org/10.1093/rheumatology/keu176

[11] Bajocchi G, La Corte R, Locaputo A, Govoni M, Trotta F. Elderly onset rheumatoid arthritis: clinical aspects.(2000). ClinExpRheumatol. 18 Suppl20:S49–50

[12] Vollenhoven Ronald F van (2009). Sex differences in rheumatoid arthritis: more than meets the eye. BMC Med;7:12. doi: 0.1186/1741-7015-7-12

[13] Kvien T.K., Uhlig T., Ødegård S. and Heiberg M.S. (2006). Epidemiological aspects of rheumatoid arthritis: the sex ratio . Ann N Y AcadSci ;1069:212-22. doi: 10.1196/annals.1351.019.

[14] Chou CT., Liao HT., Chen CH., Chen WS., Wang HP. and Su KY. (2007).The clinical application of anti-CCP in rheumatoid arthritis and other rheumatic diseases. Biomark Insights. 2: 165–171

[15] Nezam Uddin M.; Paul S.; Sattar; M.A.; Faruk G.; Kibria A; AlamgirM.,M; Biswas R.,S.,R.; Karim M.,R; Lohani K.,H.; Nath R.,K. and , Rahman M. (2017).Evaluation of anti-ccp for early diagnosis and assessment of disease severity in rheumatoid arthritis patients. International Journal of Current Research . 9(08):55762-55766

[16] Alfatlawi R., B. ; Al-Mashhadi H., Gh.; Hameed W., S. and Aljazaeri T., Dh. (2020). Comparative study between anti-ccp and rheumatoid factor as diagnostic value of rheumatoid arthritis patients . Ann Trop Med & Public .23(S6):604-608. DOI: http:// doi.org/ 10.36295 / ASRO. 2020.236

[17] Aggarwal R.; Liao K.; Nair R.; Ringold S. and Costenbader K., H. (2009).Anti-Citrullinated peptide antibody (ACPA) assays and their role in the diagnosis of rheumatoid arthritis. Arthritis Rheum. 61(11): 1472–1483.doi: 10.1002/art.24827

[18] AurrecoecheaE. ;llorcadiaz J.; Diezlizuain ML .; Mcgwin G. Jr., and Calvo-alen J. (2015). Impact of gender in the quality of life of patients with rheumatoid arthritis . J Arthritis .4:3.DOI: 10.4172/2167-7921.1000160

[19] Shafiaa S., Shaha A.A., Sofib F.A., Rasoola R. and Gulla A. (2016). Anti-CCP is Associated with Greater Disease Burden in Kashmiri Population with Rheumatoid Arthritis. Rheumatology (Sunnyvale) ,6:1 DOI: 10.4172/2161-1149.1000190

[20] Eltokhy H., M.; Ali S.,T.; Abdrabo S., A.; Gad A., M; El Sawi H.,A; Abd EL-Ghaffar N. and A Mansour M. (2011). Relationship between anti-cyclic citrullinated peptide antibodies and disease activity and extraarticular manifestations of rheumatoid arthritis in egyptian patients. AAMJ. 9(10)

[21] Shah B., G.; Quershi H., J.; ZAFAR U. (2014).Serum Anti-CCP antibody and its correlation with disease activity in local pakistani rheumatoid arthritis patients. P J M H S . 8(4):1041-1044

[22] Pawłowska J. ;Smoleńska Ż. ;Daca A. ;Witkowski J., M. ,and Bryl E. (2011).Older age of rheumatoid arthritis onset is associated with higher activation status of peripheral blood cd4+ t cells and disease activity. ClinExpImmunol. 163(2): 157–164.doi: 10.1111/j.1365-2249.2010.04294

[23] Twigg S.; Nikiphorou E.; Nam J.,L.; Hunt L.; Mankia K.; Pentony P.,E.; Freeston J.,E.; Tan AL. and Emery P. (2018) Comorbidities in anti-cyclic citrullinated peptide positive at-risk individuals do not differ from those patients with early inflammatory arthritis. Front. Med. 5(35). doi: 10.3389/fmed.2018.00035

[24] Barbarroja N.; Pérez-Sanchez C.; Ruiz-Limon P.; Castro-Villegas C.; Aguirre M.,A.;Carretero R.;Segui P.; Jimenez-Gomez Y.; Sanna M.; Rodriguez-Ariza A.; Collantes-Estevez E .; Alejandro E , and Lṕez -Pedrera Ch.(2014). Anticycliccitrullinated protein antibodies are implicated in the development of cardiovascular disease in rheumatoid arthritis. Arteriosclerosis, Thrombosis, and Vascular Biology.34:2706–2716. doi.org /10.1161/ATVBAHA.114.304475

[25] Lo´ Pez-Longo F., J.; Oliver-Min˜ Arro D.; De La Torre I.; De Ra´ Bago E., G.; Sa´ Nchez-Ramo´ N S.; Rodri´Guez-Mahou M.; Paravisini A.; Monteagudo I.; Gonza´ Lez C.,M; Garci´A-Castro M.; Casas M.,D.; and Carren˜ O L. (2009). Association between anti–cyclic citrullinated peptide antibodies and ischemic heart disease in patients with rheumatoid arthritis. Arthritis & Rheumatism (Arthritis Care & Research). 61(4): 419–424,DOI 10.1002/art.24390

[26] SanmartiR.;Gómez-Centeno A. and Ercilla G.(2007): Prognostic factors of radiographic progression in early rheumatoid arthritis: a two year prospective study after a structured therapeutic strategy using DMARDs and very low doses of glucocoticoids. Clin Rheumatol.26(7):1111-8.

[27] Fathi N., A.; Ezz-Eldin A., M.; Mosad E.; Bakry R., M.;Hamed H., B.; Ahmed S.; Mahmoud M; RashedH., Allah, G , and Abdullah F. (2008).Diagnostic performance and predictive value of rheumatoid factor, anti-cyclic-citrullinated peptide antibodies and hla-drb1 locus genes in rheumatoid arthritis. Int Arch Med.22.1(1):20. doi: 10.1186/1755-7682-1-20

[28] Ghozlani I ;Mounach A. ; Ghazi M.; Kherrab A ; Niamane R. (2018). Targeting acquired hemophilia a with rheumatoid arthritis by a rituximab shot: a case report and review of the literature . Am J Case Rep. 19: 582–588..doi: 10.12659/AJCR.908854

[29] Miriovsky B., J; Michaud K.; Thiele G., M. ; O'Dell J., R. ; Cannon G., W.; Kerr G.; Richards J. ,S.; Johnson D. ; Caplan L.; Reimold A.; Hooker R. and Mikuls T., R. (2010). Anti-CCP antibody and rheumatoid factor concentrations predict greater disease burden in U.S. veterans with rheumatoid arthritis . Ann Rheum Dis. 69(7): 1292–1297. doi:10.1136/ard.2009.122739

[30] Stropuvienė S.; Lapinienë G.; Redaitienë E. ; Kirdaitë G. ; Dadonienė J. (2005).Rheumatoid Arthritis markers: antibodies against citrullinated peptides. ACTA MEDICA LITUANICA.12(3): P. 37–41

[31] Serdaroglu M.; ÇakĬrbayH. ;Defer O. ; Cengiz S. and Kul S. (2008).The association of anti-ccp antibodies with disease activity in rheumatoid arthritis . Rheumatol Int.28:965–970