THE CORRELATION BETWEEN LEVELS OF SOME AUTO-ANTIBODIES WITH CMV AND EBV INFECTIONS IN RA PATIENTS

Hussein Hafidh Abbas*

*Ministry ofhigher education and scientific research, Al-Yarmouk University College/ Dept. of MedicalLaboratories Techniques, Baghdad, Iraq Al-Yarmouk University College / Dept. of Medical Laboratories Techniques

(Received on Date: 9th November 2016 Date of Acceptance: 29th November 2016)

ABSTRACT

Rheumatoid arthritis (RA) is a chronic inflammatory systemic autoimmune disease characterized by inflamed joints which resulted by produced of auto antibodies to cyclic citrullinated peptides (anti-CCP), Immunoglobulins (Ig) as IgG, IgM, and IgA. RA affects about 1% of the world's population; it's significantly common in females more than in males (about 3:1 female: male ratio). The pathophysiology of RA involves a complex interaction includes a complex genetic and some environmental stimulus; intra synovial immune response; and tissue injury mediated by pro-inflammatory cells, inflammatory effecter molecules, and degradation enzymes. An infectious agent is environmental factors, most notably viruses, have long been suspected of promoting the development of RA and other autoimmune diseases. EBV is one of herpes common viruses in humans, transmitted through saliva; infects and replicates into epithelial and B cells, and caused infectious mononucleosis (IM). Dysfunction of immune response may be occurred in patients with RA that resulted by elevate induction of cellular and humoral immune responses against EBV.Human Cytomegalovirus (HCMV) represents a pathogenic herpes virus, it infects 40–99% of adult populations in the world it can be infection diverse types of epithelial cells, and cause organ-specific or systemic infection. The results of the present study showed; approximately 60-80% of studied groups are positive to RF but that not directly related with RA symptom because anti-CCP antibody (ACCP) has higher levels in RA patients group than that in control group.Also, Specific IgM was formed in sera of RA patients group against CMV and EBV capsid antigen (EBVCA) showed higher levels more than control group and increased by ~10% in patients with more severe joint disease as RA than other diseases like SLE.In addition; increased serum levels of IgG, IgA occurring in RA patients; while elevated only in IgM levels in RA patients have positive of RF, that mean anti-gamma globulins were associated with clinical findings of severe rheumatoid arthritis.As a conclusion, higher prevalence of CMV IgG and IgM antibodies is found in patients with autoimmune diseases as RA, also increased levels of some auto-antibodies IgG, IgM, IgA, and ACCP with the presence of EBV infection in RA patients.

Keyword: Rheumatoid arthritis, CMV, EBV, auto-antibodies.

No:of Tables: 4 **No:of References: 23**

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory systemic autoimmune disease characterized by inflamed joints, produced of auto-antibodies, e.g., rheumatoid factors (RFs) and anti-citrullinated cycilc protein antibodies (ACCPs), and can cause systemic complications. RA affects about 1% of the world's population [1, 2]; it's an incidence of 5–50 from 100,000 people per year [3] with three times more female than male patients [4].RA can be resulted by numerous causes as stimulating of monocytes and macrophages by activated T helper (TH) cells to produce inflammatory cytokines like interleukin (IL)-1, IL-6, and tumor necrosis factor-a (TNF-a), as well as proteolytic enzymes, also send signal to B cells for produce increased levels of immunoglobulins like RF, and anti-CCP antibodies that lead to destroying synovium, cartilage, and underlying bone which developed RA. In addition; it caused by exposures to various factors as hormonal therapy, antioxidants and smoking; Sometimes it may be related with genetic factors. [5]

Among other environmental factors as infectious agents; most notably viruses as human herpes viruses, have promoting to development some autoimmune diseases as RA [6]. EBV is one of herpes common viruses in humans, transmitted through saliva; infects and replicates into epithelial and B cells, and caused infectious mononucleosis (IM) [7]. It is highly associated with several malignancies including nasopharyngeal carcinoma, Burkitt's lymphoma, T and natural killer (NK) cell lymphomas, lympho proliferative disease in immune compromised hosts, and Hodgkin's disease [5]. Autoimmune dysfunction may be occurred in patients with RA that resulted by elevate induction of cellular and humoral immune responses against EBV; like elevated of IgG antibody levels have been found against some EBV proteins [8].In addition, Human Cytomegalovirus (HCMV) represents a pathogenic herpes virus, it infects 40–99% of adult populations in the world that depending onto ethnic and socioeconomic conditions, it can be infection diverse types of epithelial cells, and cause organ-specific or systemic infection [9].Davis *et al*., 2013 [10] have shown that the presence of CMV infection may be clinical affect and outcome of RA; that occur by compartments of immune cells as NK cells, memory T cells not only in antimicrobial as antiviral immune response, but also in autoimmune response specially with presence of CMV infection when T cell mediated autoimmune disease with severe perturbations of immune homeostasis, particularly in various T lymphocyte compartments. Also similar observations have been reported in other autoimmune diseases, such as systemic lupus erythematosus [11].So, this study was investigated into related between infections some viruses as EBV and CMV to induction of some auto-antibodies like RF and CCP in serum of RA patients; and related between EBV and CMV to produce immunoglobulins like IgG, IgM, and IgA in serum of RA patients.

Material and Methods

Selection the clinical cases of RA:

Based on the clinical status of patients that investigated by consultant clinic of Baghdad Teaching hospital and serological results of RF and CCP tests for RA. A total number of RA patients were 40 plus 20 healthy person. Blood samples were taken in the period of time from October–February 2016.

Collection of clinical specimen of RA:

A total volume of blood samples collected either from each patient or control person was (5ml) by vein puncture using disposable syringes. Collected blood was allowed to clot at room temperature for 30min. and then centrifuged for 10min. at 4000 rpm, then sera were separated and divided into several aliquots tubes, one of them used for serological tests to estimate of IgG and IgM antibodies levels against CMV and EBV and detection auto-antibodies as (IgG, IgM, and IgA) levels, but other tubes were frozen at -20° C, and thawed immediately prior to analysis of ACCPs level.

Serological methods to diagnose of RA patients:

I. Qualitative determination of RF (slide latex agglutination):

Qualitative RF was detected in serum person of studied groups by slide latex agglutination method according to manufacturer instructions of (linear, Spanish), that occur by observed the positive or negative result may be found. The presence or absence of visible agglutination was observed by necked eye immediately after removing the slide from the rotator.

II. Quantitative determination of anti-CCP levels:

Anti-CCP level was measured in serum of rheumatoid arthritis patients compared to control group by enzyme-linked immune-sorbent assay (ELISA) method according to manufacturer instructions of AESKULISA®, Germany.

- Sero-quantitative method to determine of specific Anti-CMV and EBV antibodies (according to kit protocol):

Automatically Robert machine Dia Sorin/LIASION®, Italy used method for quantitative determination of specific IgG to human CMV and/or EBV into serum of studied groups by an indirect chemiluminescence immunoassay (CLIA).

Quantitative method to determine of auto-antibodies levels:

Auto-antibodies as IgA, IgM, and IgG were determined in serum of studied groups by used radial immunodiffusion method according to protocol instructions of Intermedical®, Italy.

Statistical analysis:

Results were analyzed statistically by used SPP with significance value (in 0.01 value) of difference mean between two groups was assessed by Independent group's t-test between means.

RESULTS AND DISCUSSION

Detect of RF and Anti-CCP levels in serum of RA patients:

The present study showed quantitative determines of RF levels into sera of studied groups that includes RA patients and control healthy groups. The mentioned results in table (1) was showed elevated of RF level (56.87 ± 0.34IU/ml) for RA patients group with slight significant difference ($P<0.01$) than those in the healthy control group .The results of studied groups in the above table were showed agreement of RF levels with some previous studied as Abdullah, H. N. *et al*., 2012 [12] who mentioned: elevated of RF levels as a simple parameter for RA disease activity especially during three years of onset of symptoms that associated with more severe disease [13]; Also there is association between some environment factor, long time of disease, and RF positivity ; and affect of these factors to RF produced which is one of the characteristic features of RA [14]. RF are antibodies formed directed to the FC fragment of human IgG molecules resulting in RF-IgG immune complexes which could be deposited in tissues and activate the classical complement pathway, and lead to tissue damage [15] .

Many studying showed 60-80% of patients with RA are positive to RF; but that not directly related with RA

symptoms; because RF is found significantly more often in cases of aggressive joint inflammation.

Recently, a highly specific autoantibody may be developed to citrullinated proteins that lead to form of anti-cyclic citrullinated peptide (anti-CCP) antibody [16]. The presence of ACCP is also associated with more destructive joint damage and aggressive course of the disease and involvement of ACPAs in the patho-physiology of RA.

So, the levels of Anti-CCP antibody which has been detected in the sera of the studied groups; and showed higher levels of anti-CCP antibody in RA patient's sera (200.03 ± 1.97IU/ml) rather than the control groups (3.49 ± 0.92IU/ml) with highly significant (P<0.01). These result was agree with Pruijn, G. *et al*., 2005 [17] and Abdullah, H. N. *et al*., 2012 [12] who are reported anti-CCP represents a superior serological marker for RA; because Anti-CCP is (i) highly specific for RA disease, (ii) able to distinguish RA from other arthritis that mimic RA, (iii) also present in the majority of patients (good sensitivity), (iv) detectable very early in the disease, and (v) helpful in predicting disease outcome.

Detection of anti-CMV and anti-EBV antibodies levels in serum of RA patients:

The levels of specific antibody formed in sera of studied groups was measured by chemilumencense assay to investigate their role in diagnosis of acute infection to some viruses like CMV and EBV, which remains difficult to identify from symptoms alone; also their role patho-physiology of RA, and related with action mechanism to cause RA .

So, the levels of specific IgM antibody formed against CMV and EBV capsid antigen (CA) levels in sera of RA patients compared to healthy control group was showed in table (2).

Specific IgM was formed in RA patients group against CMV and EBV capsid antigen (EBVCA) showed higher levels (58.13±0.78 and 73.9±0.56 IU/ml respectively) with highly significant (P≤ 0.01) than those in healthy control group (table 2).

These results were agreement with noticed of Bergen *et al*., 2012 [18] who was suggested sero-positivity of anti-CMV antibody in RA patients and frequency of CMV infection to be increased by ~10% in patients with more severe joint disease as RA than other diseases like SLE.

Additionally, the results in table (2) were showed elevated of specific IgM levels against EBV proteins, such as VCA in RA patients group compared to healthy controls group that agreements with several studies have shown an elevated of anti-EBV immune response in RA patients, indicating that the virus may be associated with the autoimmune dysfunction in patients with RA [19].

Detection of anti-IgG, IgA, and IgM antibodies levels in serum of RA patients:

Auto-antibodies in RA are helpful both for prognosis and diagnosis, and its association with disease severity [20]. Anti-gamma globulins of IgG, IgA and IgM antibodies were measured in studied groups by immunodiffusion method. The results in table (3) were showed; Higher elevate significantly of IgG and IgA levels in sera of RA patients group with significant (P≤ 0.01) than in healthy control; while level of IgM were simple elevated only in sera of RA patients group with slight significant (P≤ 0.01) than in healthy control.

The results in below table were agreement with Panush, R. S. *et al.;* 2000 [21] who mentioned; Increased serum levels of IgG, IgA occurring in RA patients; while elevated only in IgM levels in RA patients have positive of RF, that mean anti-gamma globulins were associated with clinical findings of severe rheumatoid arthritis .

So, the highly levels of auto-antibodies as IgG, IgA, and IgM in RA patients group could be interpreted by their role as

effective agents in prognosis and diagnosis of RA. That occurs when they produced in large amounts in synovial fluid and sera in RA patients as a resulted when ability of synovial inflammation cells like macrophages, T cells, synovial fibroblasts and B cells, which act in promoting synovial hyperplasia and degradation of cartilage and bone. It is believed that APCs in the synovial membrane present auto-antigens to T cells, which then initiate an antigen-driven immune response, characterized by macrophage infiltration and altered cytokine production [22].

Also, synovial fibroblasts are activated, adhere to cartilage constituents, and produce degrading proteinase such as matrix metallo proteinases (MMPs). In addition to this immune response, B cells encounter both the auto-antigen(s) and stimulating T cells, which promote their differentiation into plasma cells with the ability to produce auto-antibodies. So the action mechanism of auto-antibodies resulted by form immune complexes able to mediate tissue damage by complement activation or Fc receptor ligation [23].

Correlation between CMV and EBV infections with auto-antibodies levels in sera of studied groups:

This section of the present study was possible found relation between viral infections as EBV and/or CMV with RA disease. The above results in table (4) were showed highly levels of some auto-antibodies as IgG, IgA formed in sera of RA patients group who were infected either with CMV and EBV, or both of them (1314±34.91, 1121±34.9, and 1100 ±13.9 respectively) and (129.8±8.22, 95.7±6.72, and 110±3.45mg/dl respectively) with highly significant ($P \leq 0.001$) than those in the control healthy group. In additionally, the results showed higher elevated of ACCP (198.5±1.95, 187.6±1.95, and 197.9±0.96IU/ml) with significant ($P \leq 0.001$) rather than those in the control healthy group.

In contrast; the results showed equilibrium level of IgM in sera of studied groups. The higher elevated of auto-antibodies levels in sera of RA patients that agreement with Westergaard, M.W *et al*., 2015 [2] who mentioned in her studied, Increased levels of some auto-antibodies IgG, IgM, IgA, and ACCP with the presence of EBV infection in RA patients; that mean there is association between elevated and characteristics of these auto-antibodies in RA patients with the presence of EBV infected.

Also, the results agreement with Halenius, A; and Hengel, H. (2014) [9] Who said in him report; it should be expected that a higher prevalence of CMV IgG and IgM antibodies is found in patients with autoimmune diseases as RA.

So; these results administrated hypothesized there is related between viral infections as EBV infection and produced of various antibodies in RA patients that occur by simultaneous processes which are responsible for induction of some antibodies like RF, ACCP, IgG, IgA and EBVCA antibodies. These processes could be induced by recognition and destruction of EBV-infected cells, e.g., where EBV in its life cycle only expressing EBVCA in infected B cells, also these infected cells produced alteration IgG. So when destructed EBV-infected cell releasing EBVCA and IgG could be the antigen source responsible for the production of both EBNA-1 antibodies and RFs from memory B cells. [2]

Halenius, A; and Hengel, H. (2014) [9] administrated in report ability of some subtypes of T cells in strongly implicated in RA pathogenesis; actually it was observed increased of $CD4^+$ $CD28^-$ T cell population levels about 3-fold higher in CMV positive RA patients than in healthy CMV positive subjects, these increased in $CD4^+$ $CD28^-$ T cell population might be cause a significantly faster progression of

joint destruction by express granzyme B and have been shown to possess both cytotoxic activity and the ability to express IFN-γ upon encountering of HCMV antigens.

In addition, increased levels of $CD8^+$ T Cells in inflamed lesion of RA patients which positive to EBV and HCMV. Its play a role not for initiation of disease but made a preferential homing into inflamed tissue after virus reactivation and might be contributed to induced inflammation in the tissue in RA by local release of pro-inflammatory cytokines as interleukin 15 (IL-15) which is found both in the synovial fluid and serum of RA patients and has been found to attract and activate T-cells, thereby contributing to the pathogenesis of RA.

Conclusion:

Increasing significantly of RF and ACCP level in serum of RA patients with elevated of serum levels to some auto-antibodies as IgG, IgM, and IgA; and elevated of serum levels of specific IgM formed against CMV and EBVCA in serum of RA patients .

Finally, correlate significantly between levels of IgG, IgM, IgA, and ACCP antibodies with positive viral infection as CMV and EBV in RA patients that implicated in the pathogenesis of this disease.

Table (1) showed levels of RF and ACCP in sera of studied groups			
Immunity parameters	RA patients group	Healthy Control group	T- test value
	Mean ± SE	Mean ± SE	
RF IU/ml	56.87 ± 0.34	13.34 ± 1.56	2.36*
Anti-CCP IU/ml	200.03 ± 1.97	3.49 ± 0.92	1.034*
Where:*P ≤ 0.01, and SE: Standard error.			

Table (2) the mean levels of specific IgM in healthy and RA patients groups.			
Groups study	Mean ± SE.		T-test
	Specific IgM of CMV	Specific IgM of EBVCA	
RA patients group	58.13 ± 0.78	73.9 ± 0.56	12.1*
Healthy control group	2.45 ± 0.56	5.09 ± 0.34	3.49*
Where:*P ≤ 0.01, and SE: Standard error.			

Table (3) The levels of IgG, IgA, and IgM in sera of studied groups			
Immunoglobulins	RA patients group	Control group	T-test value
	Mean ± SE	Mean ± SE	
IgG mg/dl	1314±34.91	114.3±91.2	0.008
IgM mg/dl	95.7±15.05	73.5±11.8	0.076
IgA mg/dl	129.8±8.22	45.2±9.14	0.0031
Where:*P ≤ 0.01, and SE: Standard error.			

Table (4) the relation between levels of auto-antibodies with viral infections					
Parameters	RA group			Control healthy group	T-test value
	CMV	EBV	CMV/EBV		
	Mean ± SE				
IgG mg/dl	1314±34.91	1121±34.9	1100 ±13.9	114.3±91.2	8.677 **
IgM mg/dl	95.7±15.05	84.9±15.82	90.1±7.92	73.5±11.8	2.64
IgA mg/dl	129.8±8.22	95.7±6.72	110±3.45	45.2±9.14	5.79**
ACCP IU/ml	198.5±1.95	187.6±1.95	197.9±0.96	3.49 ± 0.92	1.98*
Where: (+): P ≤ 0.001, SE: Standard error, ** Highly significant, and * Significant.					

REFERENCES:

Gabriel, S.E.; Crowson, C.S.; and O'Fallon, W.M. (1999). The epidemiology of rheumatoid arthritis in Rochester, Minnesota, 1955–1985. Arthritis Rheum. 42:415-420.

Westergaard, M. W.; Draborg, A. H.; Lone Troelsen, L.; Jacobsen, S.; and Gunnar Houen G. (2015). Isotypes of Epstein-Barr Virus Antibodies in Rheumatoid Arthritis: Association with Rheumatoid Factors and Citrulline-Dependent Antibodies. BioMed Research International. 472174; Pp.:5.

Scott, D. L.; Wolfe, F.; and Huizinga, T. W. J. (2010). "Rheumatoid arthritis," The Lancet, 376 (9746): 1094–1108 .

Linos A, Worthington JW, O'Fallon WM, and Kurland LT. (2009). The epidemiology of rheumatoid arthritis in Rochester, Minnesota: a study of incidence, prevalence, and mortality. Am J Epidemiol, 111:87-98.

Karen H Costenbader and Elizabeth W Karlson. (2006). Epstein–Barr virus and rheumatoid arthritis: is there a link? Arthritis Research & Therapy. 8(1):1-7.

Toussirot, E.; and Roudier J. (2008). Epstein-Barr virus in autoimmune diseases. Best Pract Res Clin Rheumatol. 22:883-896.

H. H. Niller, H. Wolf, and J. Minarovits, (2008). "Regulation and dysregulation of Epstein-Barr virus latency: implications for the development of autoimmune diseases," Autoimmunity, 41(4): 298–328.

L¨unemann, G. D.; Frey, O.; and T. Eidner, T. (2008)." Increased frequency of EBV-specific effector memory CD8+ T cells correlates with higher viral load in rheumatoid arthritis," Journal of Immunology, 181(2): 991-1000 .

Halenius, A; and Hengel, H. (2014). Human Cytomegalovirus and Autoimmune Disease. BioMed Research International. 15:1-16.

Davis, JM; Knutson, KL; Strausbauch, MA; Green, AB; Crowson, CS, and Therneau TM, (2013). Immune response profiling in early rheumatoid arthritis: discovery of a novel interaction of treatment response with viral immunity. Arthritis Res Ther.15:199-210.

Soderberg-Naucler C. (2012). Autoimmunity induced by human cytomegalovirus in patients with systemic lupus erythematosus. Arthritis Res Ther.14:101-108.

Abdullah, H. N.; Al-Thuwani, A. N.; Nadir, M. I.; and Al-Badri, kh. (2012). Diagnostic value of Anti-CCP antibodies compared with Rheumatoid factor in Rheumatoid arthritis patients. J. of university of Anbar for pure science. 6(3): 1-6.

Jonsson, T. and Valdimarrson, H. (1993). Is measurement of rheumatoid factor isotypes clinically useful. Ann. Rheum. Dis.; 52(2):161-4.

Selman, A. N.; Mutasim, W. M.; and Al-Salih, M. W. M. (2014). Detection of Rheumatoid Factor (RF)-Iso Types and some Immunological Interactions in Patients with (Rheumatoid Arthritis -RA)in Thi-Qar Provence -Iraq. Journal of University of Thi-Qar. 9(3):55-62.

Smolen, J. (1996). Autoantibodies in rheumatoid arthritis. In: Manual of Biological Markers of Disease. Edited by van Venrooij, W. & Maini, R. Section C 1. Dordrecht: Kluwer Academic Publishers. Pp.:1-18.

Quinn, M.; Gough, A.; Green, M.; devlin, J.; Hensor, E. (2006). Anti-CCP antibodies measured at disease onset help identify

seronegative rheumatoid arthritis& predictor radiological and function outcome. Rheumatol.; 45 (4):478-480.

Pruijn, G.; Vossenaar, E.; Drijfhout, J.; Van Venrooij, W. and Zendman, A. (2005). Anti-CCP Antibody detection Facilitates early diagnosis and prognosis of rheumatoid arthritis. Current Rheum. Reviews; 1:1-7.

Bergen, V.J.; Gaalen, F.A.V.; Dongen, H.V.; Huizinga, T. W.J.; Feltkamp, M. C.; Toes, R. E. M.; and Doning, F. (2012). Anticytomegalovirus seropositivity in rheumatoid arthritis is not associated with the presence of severe extraarticular complications. J. Arithritis and Rheumatisml. 64(8):2803-2810 .

L¨unemann, J.D.; Frey, O.; and Eidner T. (2008). Increased frequency of EBV-specific effector memory CD8+ T cells correlates with higher viral load in rheumatoid arthritis," Journal of Immunology, 181(2):991-1000.

Karimifar, M., Moussavi, H.; Mehran Babaei, M.; and Akbari, M. (2014). The association of immunoglobulin A, immunoglobulin G and anti-cyclic citrullinated peptide antibodies with disease activity in seronegative rheumatoid arthritis patients. J Res Med Sci.19:823-826.

Panush, R. S.; Nicolas E. Bianco, N. E. and Schur, P. H. (2000). Serum and synovial fluid IgG, IgA and IgM anti-gamma globulins in rheumatoid arthritis. Arthritis and Rheumatism. 14 (6): 737-747.

Konttinen, Y. T.; Ainola, M; and Valleala, H. (1999). Analysis of 16 different matrix metalloproteinases (MMP-1 to MMP-20) in the synovial membrane: different profiles in trauma and rheumatoid arthritis. Ann Rheum Dis.58:691-697.

Skogh, T; Kastbom, A; and Sjöwall C. (2005). The B cells are back! Drug Discovery Today: Disease Mechanisms.2:351-357.