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Correlation the Cytomegalovirus (CMV) IgM Antibodies and Viral DNA Presence with Rheumatoid Arthritis (RA) in Iraqi Patients

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Abstract

In the Iraqi population, a high incidence (82.7%) of rheumatoid arthritis (RA) has been reported among the suspected patient. Many investigators studied the microbial infectious present in Iraqi patients with RA; however, to the best of our knowledge, there is no previous study detected the CMV DNA and antibodies to RA disease. Hence, the current study aimed to investigate the presence of CMV DNA and antibodies in Iraqi RA patients. A total of 58 blood samples were collected from patients with clinical signs of rheumatoid arthritis, along with 32 samples of apparently healthy individuals as a control group. These samples were tested for rheumatoid factor (RF), CMV IgM antibodies and viral DNA during the acute and the chronic periods of the autoimmune disease. The results showed that 46.6% (27/58) and 13.8% (8/58) of rheumatoid arthritis patients had positive reactions for IgM-CMV and CMV DNA, respectively, as compared to healthy individual. The highest rate of the viral occurrence was recorded in the aged and female RA patients (24 and 21 cases, respectively). Moreover, the most significant increases in RA appearance and CMV reactivation cases were observed in patients with a mean age higher than 40 years old. This finding pointed out the prevalence of viral infections mostly in RA patients aged more than 40 years old, accompanied by increased RA cases. This study concludes that patients with positive CMV must be tested for rheumatoid factor, especially when the viral DNA is present. Moreover, we recommend measuring TNF- α and IFN- γ as the proinflammatory cytokines that play an important role to protect against viral infection and prevent their reactivation in all RA patients.

Keywords: Cytomegalovirus, rheumatoid arthritis, ELISA, DNA amplification.

علاقة الاجسام المضادة (IgM) للفايروس المضخم للخلايا (CMV) ووجود الدنا الفايروسي مع مرضى التهاب المفاصل الرثوي من العراقيين (RA)

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الخلاصة

في المجتمع العراقي، سجلت حدوث اصابات عالية بالتهاب المفاصل الرثوي (RA) 82.7% من المرضى المشتبه فيهم. درس العديد من الباحثين الاصابات الميكروبية الموجودة في التهاب المفاصل الرثوي بالمرضى العراقيين ، ولكن حسب معرفتنا ، لم يكن هناك دراسة سابقة تكشف عن وجود الحمض النووي للفايروس

المضخم للخلايا (CMV) وازداده في مرض التهاب المفاصل الرثوي. لذا، علاقة الوجود الفيروسي هذه وثقت في الدراسة الحالية. جمعت 58 عينة دم من المرضى الذين يعانون من علامات سريرية لالتهاب المفاصل الرثوي، و 32 عينة من الأفراد الأصحاء كمجموعة سيطرة. فحصت هذه العينات للعامل الرثوي (RF)، والأجسام المضادة للفايروس CMV IgM وجزيئات الدنا (DNA) الفيروسي خلال الفترة الحادة والمزمنة لمرض المناعة الذاتية. أظهرت النتائج 46.6% (58/27) و 13.8% (58/8) من مرضى التهاب المفاصل الرثوي لديهم تفاعل موجب ل IGM-CMV و DNA CMV، على التوالي مقارنة بالأشخاص الأصحاء. سجلت أعلى معدل ظهور للفايروس في كبار السن والإناث لمرضى التهاب المفاصل الرثوي هي 24 و 21 حالة، على التوالي. فضلاً عن ذلك، لوحظت الزيادة الأكثر أهمية في ظهور RA و حالات إعادة تنشيط CMV في متوسط العمر أكثر من 40 سنة. أشارت النتائج إلى ارتفاع الإصابة الفيروسية في أغلب مرضى التهاب المفاصل الرثوية الذين تزيد أعمارهم عن 30 سنة مصحوبين بحالات زيادة في RA. استنتجت هذه الدراسة إلى وجوب تشخيص العامل الرثوي في المرضى الذين يعانون من وجود IGM-CMV، وخاصة عندما يكون الحمض النووي الفيروسي موجوداً. إضافة لذلك، يوصى بتحديد TNF- α و IFN- γ كسيتوكينات المولية الالتهابية التي تلعب دوراً مهماً للحماية من الإصابة الفيروسية ومنع إعادة تنشيطها في جميع مرضى RA.

Introduction

Human cytomegalovirus (CMV) has advert protein expression among the population throughout the phases of viral infection [1]. Therefore, the viral proteins doubtlessly modulate cellular responses inside the host. Of all the types of herpes viruses, CMV expresses the maximum number of genes that regulate innate and adaptive host immune responses [2]. The virus could typically be taken early in life and may be transferred via direct or indirect contact with infected body fluids [3].

Recent evidence proves that the energetic and latent CMV infection induces sustained systemic inflammatory reactions which might be observed using a type 1 cytokine signature [4]. It is believed that viral persistence in all inflamed individuals is chronically efficient or happens as a latent contamination wherein viral gene expression is constrained [5]. Initiation of viral replication from latency is not simply resulting from immunosuppression, but like other viruses, including HIV [6], it appears to be related to the activation of the Immune system. For example, the virus may be reactivated through tumor necrosis factor (TNF) - α , which is launched throughout the inflammation. TNF- α binds to the TNF receptor on latently infected cells, producing signals that stimulate the nuclear factor-kB (NF-kB). Consequently, the activated p65/p50-NF-kB heterodimer translocates into the nucleus and binds to the IE enhancer region of CMV, which initiates viral replication [7]. This molecular mechanism was shown to have a clinical correlation, in which the reactivation of latent CMV was connected with higher serum levels of TNF- α in patients with atopic dermatitis [8] and sepsis [9]. In addition, CMV is usually reactivated following the extreme rejection of organ transplants and after acute graft versus host disease (GVHD) in bone marrow transplant (BMT) recipients who have increased TNF- α levels [10]. Additionally, proinflammatory prostaglandins result in cyclic AMP, which then triggers viral reactivation. Stress catecholamines can stimulate the increase in cyclic AMP concentrations, because of viral reactivation. Through such mechanisms, persistent inflammation probably mediates the reactivation of CMV. Cells of the myeloid line are providers of latent CMV [11]. CMV can reactivate from latency through allogeneic induction of monocytes from seropositive donors. Viral reactivation can additionally takes place while mononuclear hematopoietic progenitors, which might be latently infected with CMV, differentiate into mature DCs. Therefore, inflammation and cellular differentiation are activities that reactivate CMV [12].

Globally, rheumatoid Arthritis (RA) is the commonest chronic systemic autoimmune disease [13]. A higher women predisposition to RA was reported, with female-to-male ratio of 3:1 [14]. The occurrence and prevalence of rheumatoid arthritis was shown to be age-dependent with a 2% lower incidence in younger than in aged population (up to 70 years). After the age of 70 years, the incidence of RA starts to decline [15].

However, it is believed that rheumatoid arthritis is virally-triggered disease. Specifically, CMV, Epstein-Barr virus (EBV). Herpes simplex virus type 1, and types of HSV1/2 were shown to be linked to the disease. Moreover, compared to single contamination with this kind of viruses, a blended infection with as a minimum of them elevated the hazard for RA at a price in keeping with a unit that could not be explicated through arthritis is an easy additive effect. CMV, by affecting lymphocytes

and monocytes, establishes a lifelong latent infection that can be reactivated periodically through immunodeficiency, with the threat of autoimmune infection [16].

In the Iraqi population, high incidence (82.7%) of rheumatoid arthritis (RA) was reported among the suspected patient [17]. Many investigators studied the microbial infections in rheumatoid arthritis Iraqi patients, however, to the best of our knowledge, there is no previous study detected the CMV DNA and antibodies to RA disease. Hence, this relationship between the existent of CMV virus and RA was reported in the present study.

Materials and Methods

Sample collection and identification

1- Study population

A total of 58 blood samples were collected from patients with clinical symptoms of rheumatoid arthritis, during the period from October 2015 to February 2016 from Baghdad Hospitals. The patients (41 females and 17 males) were selected randomly with an age range of 19-62 years (mean age 44.9 ± 11.59). In addition, thirty-two samples from apparently healthy individuals (18 females and 14 males) were selected as a control group. The samples were tested for both rheumatoid factor (RF) and CMV IgM antibodies during the acute and the chronic periods of the autoimmune disease. A detailed case investigation was filled in for all of the participants, regarding their socioeconomic status, age, gender, clinical signs, and medical history.

2-Blood sample collection

Blood samples (3 ml) were collected by disposable syringe, then placed into plain tubes which were kept at room temperature until the coagulant was formed. For serum collection, the samples were centrifuged at 3000 RPM for 5 minutes. Serum samples were dispensed in Eppendorf tubes.

3- Qualitative test for the detection of Rheumatoid Factor (RF) in the serum of patients

Spectrum RF latex reagent is a suspension of polystyrene particles sensitized with human gamma globulin. When the latex reagent was mixed with a serum containing a rheumatoid factor, visible agglutination occurred. The occurrence of the agglutination indicated that the level of RF is greater than 10IU/ml. The assay was performed according to the instructions of the manufacturing company (Egyptian Company for Biotechnology/Egypt).

4- Detection of Anti- Cytomegalovirus Antibodies

Specific IgM Estimation

All samples were stored at -20°C until tested. The tests were performed using Enzyme-Linked Immunosorbent Assay (IgM-ELISA) BioCheck kit reagents (Inc 323 Vintage Park Drive Foster City, CA 94404) to detect anti-IgM specific for CMV, in accordance with the manufacture's instructions.

5- DNA extraction

DNA was extracted from whole blood samples according to the manufacture's instructions using an AccuPrep® Genomic DNA extraction kit provided by Bioneer. The extracted DNA samples were stored at -20°C until use for conventional PCR technique.

6- Amplification and detection

The extracted DNA was amplified using specific primers for all genotypes of CMV (forward primer sequence 5'- TTTGGAGAAAACGCCGAC-3', reverse primer sequence 5'- CGCGCGCAATCGGTTTGTGTA-3' [18]). The PCR mixture was set in a total volume of 20 μl and the master mix was lyophilized to include: 1 μl of each primer, 5 μl of DNA template and 13 μl of nuclease free water. Then, the amplification program started with an initial denaturation step at 94°C for 3 min, followed by a denaturation step at 94°C for 30 sec, annealing step at 58°C for 45 sec, and extension step at 72°C for 30 sec, that were repeated for 35 cycle. Finally, an extension step was performed at 72°C for 5 min and the samples were then kept at 4°C . Specific band (about 700 bps), in gel electrophoresis with red-safe dye and 100 bps ladder, was visualized at UV-transilluminator.

Statistical Analysis

The data were analyzed using SPSS IBM version 25. The proportion and their frequencies were calculated by applying the chi-square test, cross tab and relative risk with Odds ratio to investigate the significant comparison between percentages of viral infections. One-sample T-test was used to calculate the significant differences, the mean and standard error of patient ages. P-values of ≤ 0.05 were considered statistically significant.

Results

The results showed that 46.6% (27/58) of rheumatoid arthritis patients had a reaction to IgM-CMV than a healthy individual, but no significant differences of the cytomegalovirus reactivation were present among RA patients ($\chi^2=0.28$, $P>0.05$). The highest rate of viral occurrence was reported in the aged and female RA patients (24 and 21 cases, respectively). Moreover, significant increases in RA appearance (t-test=29.5, $P<0.01$) and CMV reactivation cases (t-test=21.7, $P<0.01$) were observed in patients aged more than 40 years old, as it is summarized in Table-1.

Table 1-Effects of age on the appearance of RA and CMV reactivation among the studied patients

Age groups/ Years	No. of RA patients	Mean \pm SE	No. of CMV reactivation	Mean \pm SE
< 30	9	23.3 \pm 1.4	3	24.7 \pm 2.4
30-50	31	44.3 \pm 0.8	15	43.3 \pm 1.3
> 50	18	56.6 \pm 0.9	9	57.7 \pm 0.01
Total	58	44.9 \pm 1.5*	27	46.04 \pm 2.1*

The asterisk indicates significant increase of **RA and CMV reactivation** with the age- ($P<0.01$). Although this study showed that females are more exposed to CMV infection and reactivation in RA patients as compared to males, viral reactivation differences were not significant obtained among females ($\chi^2=0.03$, $P<0.05$) and males ($\chi^2=1.48$, $P<0.05$). Figure-1 demonstrates that the positive level of CMV-IgM was recorded in sera of more than 50% of females, but only in 35.3% of the male RA patients during this study.

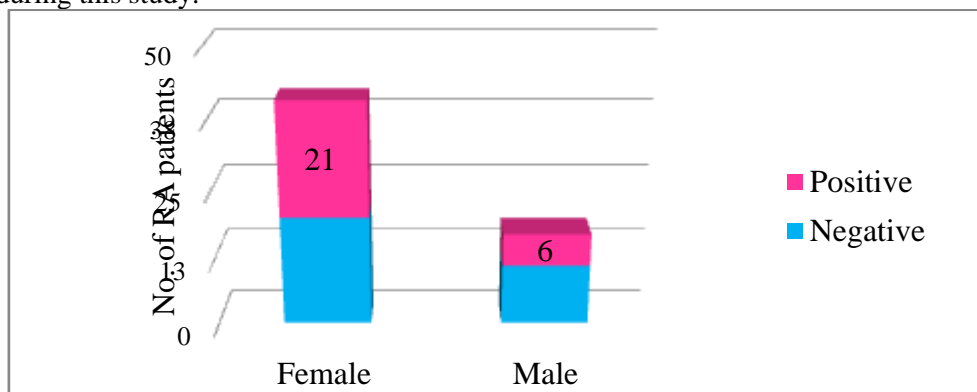


Figure 1- Positive cases of CMV-IgM among RA patients according to gender

The results also pointed out the prevalence of viral infection mostly in RA patients aged more than 30 years old, which was accompanied by increased RA cases (Figure-2). The asterisk indicates a significant incidence depended on the age of these two attributing influences.

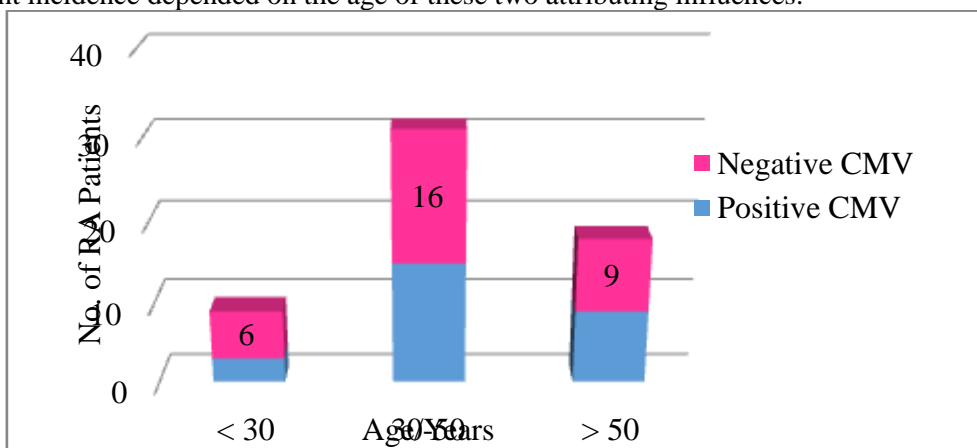


Figure 2-Distribution of CMV-IgM reactivation in different age groups among rheumatoid arthritis patients.

In the present study, the relative risk value of patients with CMV infection was 14.897 (Odds ratio=27) with more exposure to rheumatoid arthritis occurrence than those negative for the viral infection.

Regarding the detection methods, finding pointed out that type of laboratory technique for viral infection. The DNA-CMV was present in 7.4% (2/27) of IgM-positive patients, while it was detected in 19.4% (6/31) of IgM-negative patients (Figure-3).

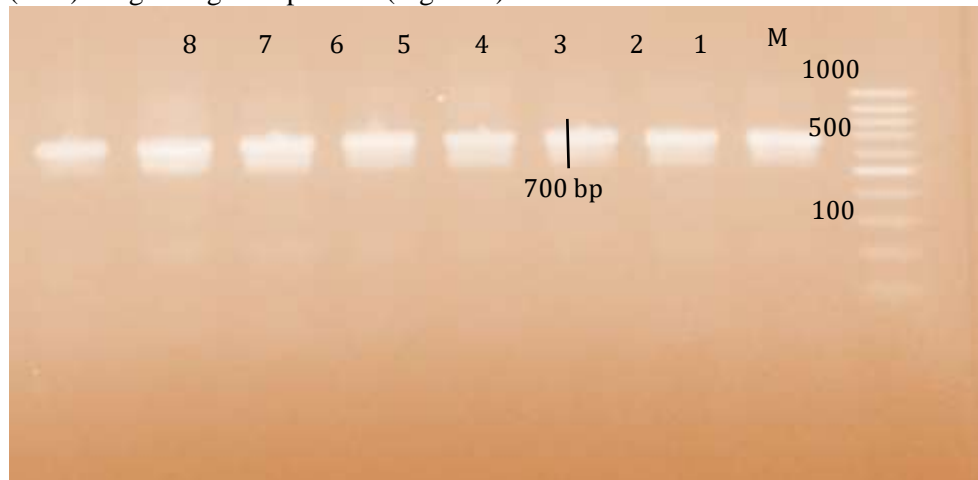


Figure 3-PCR amplification of CMV DNA from whole blood samples detected by 1.5% agarose gel electrophoresis. Lane M: 100pb molecular size marker ladder, lanes: 1-8 positive samples.

Discussion

To the best of our knowledge, no previous reports investigated CMV seroprevalence in RA patients in Iraq, although the population was reported to show higher CMV and RA incidences [19]. Remarkably, the cutting-edge look at stated that 46.6% of RA sufferers have positive CMV-IgM, this clarification that our population has 94% of CMV-IgG sera-positive [20], at the same time as the locating gave 13.8% of RA affected person have DNA-CMV as a viral particle present. A number of studies worldwide indicated the presence of CMV DNA, particular antigens, and infectious virus debris in the synovial tissue and fluid from the joints of 10% to 50% patients with rheumatoid arthritis [21].

A positive serum anti-CMV IgM result may not differentiate between primary contamination and reactivation of latent disease [22]. In addition, qualitative testing methods have variable sensitivity, and reliability may additionally varies between the commonly used diagnostic kits. Furthermore, false-positive anti-CMV IgM antibodies can occur within the presence of rheumatoid factors or heterophile antibodies [23, 24]. Quantitative assessment of viral DNA reproduction can be estimated through PCR techniques. PCR should be used to verify CMV contamination while qualitative CMV is checking out (IgM) is superb, and similar to CMV pp65 antigen trying out, PCR outcomes are in particular beneficial for diagnosis, determining the want for treatment initiation and for tracking response to therapy [22]. Unfortunately, there is no absolute threshold for the variety of DNA copies which could differentiate active as opposed to latent contamination, for that reason this take a look at is most useful for monitoring viral burden and reaction to remedy over time [25, 26].

Among person women, hormonal risk elements for RA include age at menarche, progestin use, oral contraceptive use, termination of being pregnant, lactation, and short fertility duration [27]. There are several other factors which can make contributions, such as genetics and the social life style, In addition, the sickness will also be slowly progressive and uncommon in female female with AS [28]. Differences in intrinsic factors (i.e., genetic, hormonal, gender, and different phenotypic variations) or extrinsic factors (i.e., society- or subculture-related differences in a physical activities, postpone in diagnosis, environmental influences, infections, and smoking) or an aggregate of each could contribute to this different risk of developing RA [29].

Several hypotheses have been developed in respect to the causes behind RA. First, RA may develop additionally as an end result from a polyviral network or the cumulative influence of several microbial/viral elements. Second, the RA may progress from preclinical to the late-degree disorder

due to cumulative episodes of viral infections. Third, viral elements triggering RA may be affected by different factors that have impact on the individual's health, including tobacco, ethnic conflicts, mental strain, pleasure, or continual joint tissue micro-damages. On the other hand, others consider that RA development occurs even with normal contamination frequency, period and outcomes of an immune allergic reaction to viral infections, that may result in loss of tolerance to self-antigens[30].

In addition to viral infections, there is an overexpression of epidermal growth factor receptor (EGFR) gene in the synovial tissue cells, and bone marrow-derived mononuclear cells were also linked to RA. The overexpression of EGFR in RA cases was shown to be associated with a single nucleotide polymorphism (SNP) of this gene [31]. Overall, it has to be noted that cell reception of viruses, including herpes virus, can contribute to the RA development. In addition, younger RA patients were linked to preceding infections often more than older ones [32].

Conclusion

This study concluded that patients with positive CMV must be tested for rheumatoid factor, especially when the viral DNA present. Moreover, we recommend investigating the impact of proinflammatory cytokines such as TNF- α and IFN- γ in the protection against viral infection and reactivation in all RA patients.

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