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Serum and Synovial Fluid Markers of Helicobacter Pylori Infection in Rheumatoid Arthritis and Osteoarthritis Patients

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Introduction

Recently, several infectious agents including Epstein-Barr virus and Escherichia coli have been suggested as a possible contributing factor to pathogenesis of Rheumatoid arthritis (RA). Viral agents, alone or together with bacterial pathogens, can either directly or indirectly through induction of autoimmune reactions can damage joints. Several previous studies have evaluated an association between H. pylori and development of RA, while some of this studies could establish an association between H. pylori infection and elevated disease markers of RA (1), there's also a cohort study with a relatively large sample size which did not find a difference in risk of acquiring RA between H. pylori positive and negative patients in a follow-up period of 8 years (2). Due to controversial findings in the literature on the relationship between H. pylori infection and autoimmune disease, this study aimed to compare the serum markers of infection with HSV1 and Helicobacter pylori between RA patients and OA patients as controls.

Methods

This retrospective case-control study was conducted on OA and RA patients who referred to Rheumatic Diseases Research Center (RDRC) affiliated to Mashhad University of Medical Sciences, Mashhad, Iran, from March 2015 to 2016. In general, 200 patients were randomly selected to participant in this study (100 RA cases and 100 OA patients). We excluded patients who were suspected of having septic arthritis, had crystals on microscopic evaluation of their synovial fluid, and consumed any form of antibiotic during two weeks prior to sampling, and patients with no available medical records or a history of herpes labialis during 3 months prior to sampling. The data were gathered by a researcher-made questionnaire covering demographic information, which was completed for each case separately. Synovial fluid aspiration (2-5 milliliters) was performed by a rheumatology subspecialist using sterile aspiration needles and sterile tubes with no additives and were stored at -20°C at appropriate laboratory conditions to be analyzed later. All synovial fluid samples were evaluated for crystalopathies and infections and suspicious cases were excluded from the study. The remaining samples were centrifuged at 12000 g for 30 minutes and were stored at -20°C. All laboratory operators were blinded to clinical diagnosis. In 2018, 26 individuals from RA group and 25 individuals from OA group attended a follow-up session during which a venous blood sample (50-10 milliliters) was taken by a laboratory operator using sterile needle. The obtained samples were centrifuged at 1200 g for 10-15 minutes and stored at -20°C to be later analyzed for serum markers of infection. Real-time PCR (RT-PCR) for HSV1 and 2 and H. pylori's DNA: After DNA extraction, RT-PCR was performed using Fanavari Novin kit (Tehran, Iran) according to the manufacturer protocol. Enzyme-linked immunosorbent assay (ELISA) for serum markers of HSV1 and 2 and H. pylori infection was performed using a Pishtaz Teb Zaman Diagnostics (Tehran, Iran) ELISA kit. This study was approved by the Ethics Committee of Mashhad University of Medical Sciences, faculty of medicine which can be accessed using the code "IR.MUMS.sm.REC.1395.614". Statistical analyses were carried out using IBM-SPSS software version 21. After the calculation of the normality of data, t-test or its nonparametric equivalent test (i.e., Mann-Whitney U tests) was employed to analyze the variables. A p-value less than 0.05 was considered statistically significant.

Results

Totally of 200 sample the female was 161 (80.5%) and male was 39 (19.5%). The participation were matched in terms of gender and disease duration. The age of patients in the osteoarthritis group was significantly older than those in the rheumatoid arthritis group (P <0.001).
Serological data of patients with rheumatoid arthritis showed that the mean of ESR was 34.18±2.57 mm/h and anti-CCP was 122.10±1.59 IU/ml. The anti-CCP parameter in 82% and ESR in 58% was higher than normal and the rheumatoid factor (RF) was positive in 77% of patients in the rheumatoid arthritis group. All samples were herpes virus DNA negative and the two of synovial fluid sample was positive for Helicobacter pylori's. The statistical analysis of data show that no statistically difference was found between the HSV IgG level in RA and OA groups (P=0.66), RF-negative and -positive patients (P=0.21), positive and negative anti-CCP groups (P=0.205), as well as positive and negative ESR groups (P=0.13). However no statistically significant difference female RA patients and their OA counterparts (P=0.67) and male RA patients and their OA counterpart (P=0.82). The other obtained results showed that the positive H. pylori's serum IgG condition was no statistically significant difference between two groups (P=0.82). No statistically significant difference was observed between two groups in terms of H. pylori's serum IgA (P=0.89) and H. pylori's serum IgM (P=0.13). Table 1 presents the two groups in terms of positive H. pylori's serum IgG, IgA, and IgM. The H. pylori's serum IgG levels was positive in 72.7% in RF-positive patients and 27.3% in RF-negative patients and there was no statistically significant difference between these two groups (P=0.589) based on IgG levels.

Table1: Comparison between two groups in terms of positive H. pylori's serum IgG, IgA, and IgM

Variables	RA group	OA group	*P*-value
Serum H. pylori IgG (IU/ml)	50%	48%	0.82
Serum H. pylori IgA (IU/ml)	34.6%	40%	0.890
Serum H. pylori IgM (IU/ml)	30.8%	12%	0.130

Conclusion

Our study shows that there is not a significant difference for infection with HSV1, 2 or H. pylori between RA and OA patients, making it unlikely that these two agents have an important role in development of RA. The results of previous studies on this field have been mixed. For instance, in a large cohort study by Bartels et al., researchers evaluated a total of 56000 patients for H. pylori using urea breath test (UBT) and these patients underwent a follow-up period of 8 years. Their results indicate that there was no significant difference in terms of incidence of new RA case between H. pylori positive and negative patients during the course of that follow-up period (2). Similar to our study, no difference was reported in clinical and laboratory findings between RA patients with H. pylori positive and those aith H. pylori negative except for CRP (3). Burgos et al. showed that IgG and IgM levels were reported to be above normal in 50% of patients by ELISA. Analysis of IgG and IgM antibodies by ELISA method in serum and joint fluid of patients simultaneously showed that there was no significant difference between the level of antibody and PCR (4). This study showed that the presence of herpes simplex virus DNA in the synovial space in one third of patients with rheumatoid arthritis could be due to the patient's temporary exposure to herpes simplex virus. In our study, PCR of herpes simplex virus types 1 and 2 was not positive in any of the joint fluid samples of patients with rheumatoid arthritis and osteoarthritis. In the above study, no trace of herpes virus was reported in joint fluid and serum samples of patients with osteoarthritis; therefore, it can be shown that both studies have been conducted in a single direction. Moreover, the results of our study is also consistent with a study done by Us et al. on 87 RA patients, in which they found that there was no significant difference for seropositivity for HSV1 and 2 between RA patients and the control group (5).
The key limitation of the current study was the lack of follow-up after the commencement of H. pylori eradication therapy to assess clinical outcome of RA patients who were seropositive for this agent. We suggest future studies to include a follow-up in long enough intervals after eradication therapy and evaluate how RA patients have responded when compared to their baseline. The results of our study suggests that since the difference between the level of the serum markers and OA patients as controls is not significant and that the DNA particles of both agents are absent in the synovial fluid of RA patients, it's unlikely that either HSV1, 2 or H. pylori have a role in the development of RA.

Keywords

Rheumatoid arthritis; Osteoarthritis; Herpes simplex virus; Helicobacter pylori; Synovial Fluid

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