Current Research in Microbiology and Biotechnology
Vol. 5, No. 5 (2017): 1254-1257
Research Article
Open Access

ISSN: 2320-2246

Serum and Urine level of Interlukin-8 in Iraqi Reactive Arthritis Patients

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Received: 19 September 2017 Accepted: 04 October 2017 Online: 09 October 2017

ABSTRACT

Reactive arthritis (ReA) is defined as arthritis occurring following an infection despite the inability to culture pathogens from fluid extracted from the affected joints, it belongs to spondyloarthritis (SPA). This study down to assess the relationship between reactive arthritis and urinary tract infection , and for detecting of the immune parameter level of IL-8 and determining the relation between any change in their level and UTI infection. The study included 75 Iraqi patients with reactive arthritis, and 30 healthy controls with the age range between 20 - 60 years. The Vitek-2 compact was used to identify the bacterial culture the isolate from urine of 75 patients. The results showed the *Escherichia coli* was recorded 38.66% while *Klebsiella pneumonia* 17.33%. Therefore *Escherichia coli* may be one of causes of urinary tract infection which induced reactive arthritis and urine examination and culture of urine should be done as routine in diagnosis and reactive arthritis patients. Also the our results showed that serum and urine levels of IL-8 in ReA patients were not significantly different from the normal controls, but there is significant difference noted between the serum level of IL-8 in reactive arthritis patients UTI +ve, UTI-ve and urine level reactive arthritis patients UTI +ve, UTI-ve. However, their levels were higher in serum than in urine.

Keywords: Reactive arthritis, urinary tract infection, cytokines.

1. INTRODUCTION

Reactive arthritis (ReA) is a sterile synovitis occurs after a true infection in the genitourinary or gastrointestinal tract. Environmental exposures have been implicated as potential causes for approximately all chronic diseases, Reactive arthritis is one of these diseases that caused by a known bacterial effect. Also ReA refer to the immune-mediated synovitis occurring by slow bacterial infections and detected by finding of bacteria compounds and/ or immunogenic bacterial antigens that release by metabolically activity of bacteria in the joint or another site of the body [9, 23]. Arthritis classified into the group of arthritides is called the spondyloarthropathies (SpA), ReA may be developed within 2–4 weeks of the occurring gastrointestinal or genitourinary infections. Also, SpA includes ankylosing spondylitis (AS), psoriatic arthritis (PSA), arthritis related to inflammatory bowel disease (IBD-SpA) and undifferentiated SpA. ReA is effected on one or more joints and it can induced by inflammation of the tendons, eyes, genitourinary tract and skin and it occur as an autoimmune condition with a reaction against an infection in anywhere from the body [24]. The most well-known enteric bacterial pathogens that have been involved in ReA include *Salmonella, Shigella,* and *Campylobacter* [27]. The great microscopic organisms related with ReA are Gram negative obligate or facultative intracellular aerobic microbes with a lipopolysaccharide-containing outer membrane, the essential concentration of disease is believed to be through the bodily fluid layer, either in the gut or in the urogenital tract [21]. Host and bacterial cooperation's assume a basic part in the pathogenesis of ReA, a mix of bacterial ingenuity in tissues and host helplessness factors brings about the advancement of joint irritation [21]. ReA have two form, the first one is called HLA-B27 non-associated

ReA (bacterial arthritis).[7,10,13]. And the second one is called HLA-B27 associated ReA, this type dependent on the immunogenic marker called HLA-B27 [3,11]. Unique features that can be considered as psoriasis form and in a lot of cases may be resemble these in pustular psoriasis characterize ReA, and the articular manifestations may be similar with these of psoriatic arthritis. Another important feature of ReA that similar with some cases of guttate psoriasis, ReA is a post-infectious entity and therefore the theory of molecular mimicry may have a place in the pathogenesis of both entities. This theory provided a good explanation about the immune system and how can lose tolerance in host tissue then the infection occur and increase. Lipopolysaccharides (LPS) and nucleic acids from the microscopic organisms have been confined from influenced joints, proposing that bacterial antigens may have an immediate part in the pathogenesis of ReA [26]. Many Studies showing the relation between pathogens that induced ReA and human peptides [5]. IL-8 was identified as a neutrophil-specific chemotactic factor for leukocytes with pro inflammatory and growth-promoting activities and later classified as inflammatory cysteine-any amino acid-cysteine (CXC) chemokine member of the CXC chemokine family. IL-8 is produced by a variety of cells, such as monocytes and macrophages, neutrophils, lymphocytes, and endothelial and epithelial cells, after stimulation with IL-1a, IL-1b, IL-17, TNF-a, or TLRs [4]. The receptors for IL-8 are CXCR1 (IL-8RA) and CXCR2 (IL-8RB) [12]. IL-8 has two primary functions, it induces chemo taxis in target cells, primarily neutrophils but also other granulocyte, causing them to migrate towards the site of infection [2].

2. MATERIALS AND METHODS

2.1 Patients group

This study was done on 75 (55 females, 20 males) Iraqi Reactive Arthritis, aged from (20-60) years who were referred to the Consultant Clinic at the Department of Rheumatology, Baghdad Teaching Hospital during the period November 2016- March 2017 .The diagnosis was made by the consultant medical staff at the clinic, according to ACR criteria with aid of laboratory diagnosis (ESR, CRP, RF).

2.2 Control group

Thirty apparently healthy persons male and female who matched with patients for age and gender were selected. It is ensured that they have no history or clinical evidence of reactive arthritis or any chronic disease or autoimmune disease and obvious abnormalities.

2.3 Laboratory methods

2.3.1 Blood sample collection

Seven milliliter of venous blood sample with drawn from each subject under aseptic technique. Three milliliter of each samples were transferred to test tube, centrifuged at 25000 rpm for 10 minutes and separated serum was divided into aliquots and immediately frozen at -20 ºC till further use, for CRP and RF evaluation and for assessment of cytokines . The second aliquot (2 ml) of blood is placed in EDTA tube to prevent clotting for ESR test.

2.3.2 Urine Samples collection

The diagnosis of urinary tract pathogens is depended on the quantitation of bacteria in the urine. The culture was taken from midstream urine into a sterile wide-mouth container [15] and females were instructed to wash their outer genitalia with water before a specimen collection.. Blood agar and MacConkey agar were used for the culture of urine samples. All samples were collected and transported to the laboratory within an hour by using a cool box, because low temperature inhibits bacterial multiplication in the urine samples until processed in laboratory. The urine samples were examined and cultured in less than two hours. (2ml) of urine sample centrifuged and frozen for assessment of cytokines.

2.3.3 General examination of urine sample.

Urine sample was examined by light microscope (General urine examination), then it was cultured. In the first evaluation, (5ml) of urine sample was transferred into centrifuge tube and centrifuged at 3000 rpm for 10 minutes .One drop of the sediment was placed on a glass slide and covered with a cover slip, and then it was examined by high power objective lens (40x). Then examined different field for leukocytes [22]. The urine was mixed well and the loop was inserted into the urine vertically to allow urine to adhere to the loop. Then a loopful of urine was spread uniformly on the surface of blood agar plate and McConkey agar plate, and then, all plates incubated for 24 hr at 37°C.

2.3.4 VITEK 2 system for identification of bacteria.

2.3.4.1 Principle

The VITEK 2 compact system is a fully automated system that performs bacterial identification by biochemical analysis using colorimeters and susceptibility testing of clinically significant bacteria using the VITEK 2 Product line of cards.. The VITEK 2 compact system is highly automated and allows for the rapid, accurate identification of some bacterial strains in as little as two hours. In addition to being able to identify bacteria, the VITEK 2 compact system is able to identify multiple species of yeast. In total, the system's database is capable of identifying a variety of microorganisms. The system consist 64 wells; each well is found inside a dehydrated medium and colorimetric reagents used to identify the spore-forming Gram-positive bacilli and gram negative bacteria. The other colorimetric reagent cards apply to all system formats for both industrial and clinical laboratories.

2.3.5 Estimation serum and Urine level of IL-8

The sera and urine of patients and controls were assessed for the level of IL-8 by means of enzyme linked immunosorbent assay (ELISA) method using commercially available kit (PeproTech; USA).

2.4 Statistical analysis

Frequency of UTI was given as a percentage, while cytokine data were presented as mean ± S.E., and differences between means were assessed by Duncan's test. The difference was considered significant when the probability (P) value was ≤ 0.05. The package SPSS version 13 was employed in these analyses.

3. RESULTS AND DISCUSSION

The present study showed that female 73.33% had the higher percentage than male 29.33 % as in figure 1.

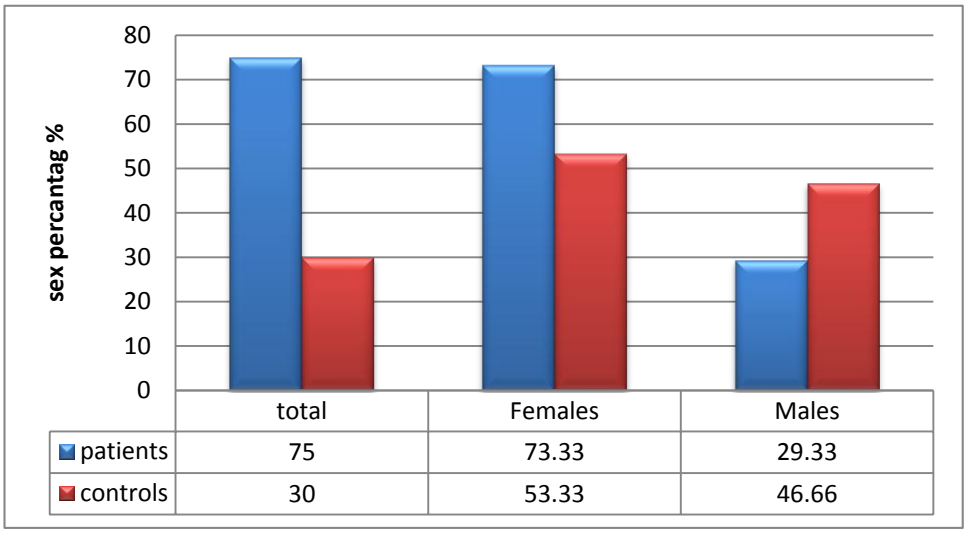

Figure 1: The percentage distributions of reactive arthritis patients and control according to gender

In this study, the high percentage of ReA patients was in 21-40 years which represent 39.9% and was in 50-70 years which represent 32.6% in composed with 41-50 years the percentage of ReA patients was 17.3% and ReA was noted have single peak incidence at 27 - 34 years [17]. The results was agreed with others studies we suggested that the reactive arthritis affects people in the second to fourth decades of life, and concluded that reactive arthritis mostly attacks young ages 20-40 years old [1,14] , while over the age of 50 and below the age of 20 is rarely seen and also Mathila *et al*., (2003) was noted the patients with ReA were adults age range 40 - 47 years [18] . ReA is most common in young men and It rarely occurs in children; when it does, the enteric form of the disease is predominant. Most pediatric patients present with symptoms after the age of 9 years [8,20,25] . Laboratory diagnosis that of all seronegative patients for rheumatoid factor (RF) , seropositive C-reactive protein (CRP) These agreed other studies that was CRP positive and RF negative [19], and the results showed there was elevated erythrocyte sedimentation rate (35 _55 mm/hr) in comparison with the control (0- 25 mm/hr) , the ESR is important in the diagnosis of inflammatory conditions and in the prognosis of non-inflammatory conditions [16]. The results of present study showed Out of 75 systemic autoimmunity patients with reactive arthritis , 56.6 % were observed to have UTI , *E. coli* and *Klebsiella pneumonia* this pathogens they were identified as a cause of UTI in the investigated patients and controls. In total autoimmunity ReA patients, *E. coli* was present as a single causative pathogen in 38.66 % of patients, while the corresponding percentage frequency for *Klebsiella pneumonia* was 17.33%. In addition, 6.6% of patients showed mixed infection of *E. coli* and *Klebsiella pneumonia* and this results agree with Farrell *et al.,* (2003) who showed the *E. coli* it was recorded the predominant pathogen (56.3-77.3%) and the next *Enterococcus faecalis*, *Klebsiella pneumonia* and *Proteus mirabilis* were important which differences in prevalence from category to other [6]. In the control patients *E. coli* represented 13.2% as in Table (1).

Table 1: Percentage frequency of urinary tract infection in total autoimmunity reactive arthritis patients and controls.

Groups	Total NO	Total NO E. coli	%	Total NO Klebsiella	%	Total NO Mix	%
Patients	75	29	38.66	13	17.33	5	6.6
controls	30	4	13.2	0	0	1	3.3

Serum and urine level of IL-8 was assessed in 66 of reactive arthritis patients had urinary tract infection ,as was as 22 apparently healthy control and the results showed no significant deference between reactive arthritis patients serum and urine UTI +ve and UTI-ve as compared with control serum and urine

UTI+ve and UTI-ve , but there is a difference noted between serum level of IL-8 in reactive arthritis patients serum UTI +ve , UTI-ve and reactive arthritis patients urine UTI +ve ,UTI-ve as in Table (2).

Table 2: Serum and urine levels of interleukin-8 in reactive arthritis patients and controls

Groups	Number	Mean IL-8 Level ± S.E. (pg/ml)
Reactive Arthritis UTI+ve (Serum)	42	40.49 ± 0.36B
Reactive Arthritis UTI-ve (Serum)	24	40.30 ± 0.38B
Reactive Arthritis UTI+ve (Urine)	42	28.98 ± 0.28D
Reactive Arthritis UTI-ve (Urine)	24	29.40 ± 0.78CD
Control UTI+ve (Serum)	4	41.35 ± 1.69AB
Control UTI-ve (Serum)	18	42.99 ± 0.72A
Control UTI+ve (Urine)	4	29.78 ± 1.01CD
Control UTI-ve (Urine)	18	31.33 ± 0.40C

Similar letters: No significant difference ($p > 0.05$) between means (Duncan test)
Different letters: Significant difference ($p \leq 0.05$) between means (Duncan test)

4. CONCLUSION

The urinary tract infection can be considered as a risk factor for ReA, and the most frequent pathogen was *E. coli* followed by *Klebsiella pneumoniae* , also the investigated interleukins IL-8 might have no effect on the pathogenesis of ReA. However, their levels were higher in serum than in urine

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