Assessment of Urinary Tract Infection and Anti Lactoferrin Antibodies Levels in Iraqi Patients with Rheumatoid Arthritis

تقييم خمج المجاري البولية ومستويات اضداد اللاكتوفيرين المضادة لدى المرضى العراقيين المصابين بإلتهاب المفاصل الرثياني

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Abstract

Rheumatoid arthritis (RA) is an autoimmune disease that may be triggered by urinary tract infection (UTI) especially if they are investigated in the presence of lactoferrin antibodies. The objective is to determine anti lactoferrin antibodies in RA and its association with urinary tract infection. Out of 162 autoimmunity Iraqi patients, UTI and levels of anti lactoferrin antibodies were assessed in 98 (60.49 %) with rheumatoid arthritis (RA), compared with 30 apparently healthy control and 74.48 % were observed to have UTI, the predominant bacteria identified as a cause of UTI; *Proteus spp*. was present as a single causative pathogen in 58.90 %, while *E. coli* was observed in 17.80 %. Anti lactoferrin antibodies were significantly higher in RA patients was observed in UTI+ve versus UTI-ve cases in total patients compared with healthy control that P value (≤0.05).

Key words: Urinary tract infections, rheumatoid arthritis, anti lactoferrin antibodies.

الملخص

التهاب المفاصل الرثياني من أمراض المناعية الذاتية التي تحفز من خلال التهاب المجاري البولية خاصة عند وجود اضداد اللاكتوفيرين. من مجموع 162 مريض مصاب بالامراض المناعية الذاتية.عزلت الجرثومة المسببة لخمج المجاري البولية وقياس مستوى اضداد اللاكتوفيرين في 98 (60,49%) مريض مصاب بالتهاب المفاصل الرثياني ومقارنته مع 30 شخصا يبدون أصحاء بلغت نسبة خمج المجاري البولية 74,48% من المجموع الكلي لمرضى التهاب المفاصل الرثياني بلغت نسبة عزلات البروتيس 58,90 % و الايشرشيا القولونية 17.80% مريض وايضا وجد ارتفاع ملحوظ في نسبة أضداد اللاكتوفيرين في مرضى التهاب المفاصل الرثياني المصحوب بخمج المجاري البولية مقارنة بالتهاب المفاصل الرثياني غير المصحوب بالخمج وكذلك بالمقارنة بالسيطرة (P≤0.05).

الكلمات الدالة: خمج المجاري البولية, التهاب المفاصل الرثياني , أضداد اللا كتوفرين

Introduction

Rheumatoid arthritis (RA) is a potentially disabling chronic systemic polyarthropathy with a world-wide distribution and an increased likelihood to have a considerable amount of negative impacts on the economic status of the patient and society [1].The cause of this disease is generally agreed to be due to a combined action of genetic and environmental (mainly microbial) factors [2].

Among the urologists and rheumatologists, the evidence of the link between UTIs and RA is not apparently recognized because of the consistent lack of data supporting this association and more probably because of the possibility for an existing hidden infection expressed in the form of asymptomatic bacteriuria in patients with RA [1].

Urinary tract infections (UTI) are among the most common infections in humans, occurring predominantly in females of all ages. *Escherichia coli* are the most common pathogen, causing 80% of uncomplicated UTI [3]. Anti-neutrophil cytoplasmic antibodies (ANCAs) are a group of autoantibodies, mainly of the IgG type, against antigens in the cytoplasm of neutrophil granulocytes and monocytes. They are detected in a number of autoimmune disorders against, established target antigens like Proteinase 3, Myeloperoxidase, Lactoferrin (LF), Lysozyme, Elastase, Cathepsin G, and Azurocidin [4].

Lactoferrin (LF) is a multifunctional immunoregulatory protein that has been associated with host defense at mucosal surfaces through its antibacterial properties. The importances of bacterial infection in systemic immunity, RA patients have greater risk to develop different infections, including urinary

tract infections (UTI) [5]. Lactoferrin (LF) which belongs to the ANCA is an iron-binding protein, which occurs in high concentrations in secretions at mucosa surfaces, in tears and in milk and urine. The antibacterial and anti-inflammatory properties of LF were further explored with an animal model of experimental Urinary tract infection [6]. Inflammation of small-sized blood vessels is a hallmark of Rheumatoid arthritis is the most common inflammatory form of arthritis, LF antibodies levels were measured as they have possible role in pathogenesis and in active disease expression especially with urinary tract infection [6].

LF also resides in the specific granules of polymorphnuclear neutrophil leukocytes (PMN) and becomes exocytosed upon PMN activation during active inflammatory disease. Hypothetically, LF-ANCA could therefore have pathogenic importance by counteracting the anti-inflammatory effect of LF antibody [5]. Lactoferrin is an iron-storing protein from the specific granules of the neutrophil granulocytes. It binds the free iron ions that bacteria need for their growth and thus has an antimicrobial effect; this protein is found in milk, tears, and urine [4]. Lactoferrin is a powerful antimicrobial able to inhibit a wide range of pathogenic bacteria and other microbes [7].

Aims of Study

Detection anti lactoferrin antibodies in RA and its association with urinary tract infection.

Materiales and Methodes

Out of 162 systemic autoimmunity patients**,** who were referred to the Consultant Clinic at the Department of Rheumatology, Baghdad Teaching Hospital during the period October 2013-September 2014 for diagnosis and treatment by physician. After a clinical examination and laboratory investigations the consultant diagnosed RA patients (98 cases: 64 males and 34 females). The S.E. was 40.1±14.39 for male and, 39.9±2.3 for female compared with 30 apparently healthy controls of blood donors matched. The means age of patients ±S.E was 40.1±1.4 compared to apparently healthy controls.

From each individual 5ml of venous blood was collected and divided into several 0.5 aliquots and all frozen at -20 □□C till used. Anti Lactoferrin Ab, RF was detected by enzyme-linked immunosorbent assay (ELISA) technique company (IMMUCHEM-France).

For detection of urinary tract infection subjects were instructed to collect mid-stream urine into a sterile wide-mouth container, and females were instructed to wash their outer genitalia with water before a specimen collection.

Each urine specimen was first examined microscopically, and then it was cultured. In the first evaluation, 10 ml of urine sample was centrifuged at 3000 rpm for 10 minutes, and the deposit was examined by high power objective lens (40x). At least ten high power fields were examined for the presence of leukocytes.Such evaluation was considered preliminary, because the judgment of UTI positivity was based on culture findings.

The number of microorganisms per milliliter recovered from urine culture can aid in the differential diagnosis of urinary tract infection. A loopful of urine (0.01 ml) was spread uniformly on Positive cultures were further identified for members of the family *Enterobacteriaceae* by methods that included morphological identification (MacConkey agar and Eosin Methylene Blue) and biochemical tests (oxidase, indole formation, methyl red, Voges-Proskuar, catalase, citrate utilization, urease, gelatinase and motility tests), as well as confirmatory AP I20 E test.

The student T test and chi-square test were used to compare soluble factor level among patients and control group and to test for associations between variables. (p-value of 0.05) or less was designated as significant.

Results and Discussion

Out of 162 systemic autoimmunity patients,.74.48% were observed to have UTI, and such frequency was 60.49% in RA patients while RA patients without UTI represent 25.51%, these frequencies were higher than the recorded frequency in controls(18.5%) Table (1).

Table (1): Percentage frequency of urinary tract infection in total autoimmunity, rheumatoid arthritis patients and controls.

Total Autoimmune disease 162	Patient with RA disease		UTI		Healthy control
	No.	%	UTI+ve No. (%)	UTI_ve No. (%)	No. (%)
	98	60.49	73(74.48%)	25(25.51%)	30(18.5%)

Proteus spp. was present as a predominant causative pathogen in 58.90 % of patients, while the corresponding percentage frequency for *E.coli* was 17.80 %. In addition12.32% of patients showed *Klebsiella spp.*, 6.84 % *Enterobacter spp.* and 4.10 % of *Enterococcus fecalis* in total of 73 UTI patients with RA. Table (2).

Table (2): Laboratory findinding of UTI in patients with RA disease

	Bacteria spp. NO.=73	NO.	%
1	*Proteus spp.*	43	58.90 %
2	*E.coli*	13	17.80 %
3	*Klebsiella spp.*	9	12.32%
4	*Enterobacter spp.*	5	6.84 %
5	*Enterococcus fecalis*	3	4.10 %
	Total	73	100%

The anti lactoferrin antibodies was found more than 10 U/ml in 64(87.67 %) rheumatiod arthritis with urinary tract infection patients but not in RA without urinary tract infection with higher significant (p value≤ 0.05) Table (3).

Table (3): Comparison of Anti-lactoferrin antibodies levels between RA patients with UTI and those with RA without UTI

Anti-lactoferrin antibodies	RA Patients UTI +ve NO.=73		RA UTI-ve NO.=25		*P ≤
Positive(≥10U\ml)	No.	%	No.	%	0.001
	64	87.67	2	8	
Negative(<10 U\ml)	9	12,32	23	92	0.001

P≤0.05 Significant

Serum anti LF antibodies concentration show significant differences p ≤0.001 in RA patients with UTI compared with RA patient without UTI and healthy control but no significant differences between healthy control and RA patient without UTI that each value is the mean±SEM Probability of Significance Differences Table (4)

Table (4): LF antibodies concentration in healthy, RA patients with and without UTI

Study population	NO.	F antibodies conc.(ng\ml)
Healthy	30	3.9070±1.0620
RA patients with UTI positive	73	58.3±14.9
RA patients with UTI negative	25	4.300.0±646.3

The last table (5) explains the association between lactoferrin antibodies means concentration in RA patients with urinary tract infection in comparison to healthy control with a significant difference ($p \leq 0.05$ value).

Table 5: Anti lactoferrin Ab level in RA patients with urinary tract infection in comparison to healthy control

Statistical analysis	RA with UTI	Healthy control	P-value
Mean	19.47±8.5	3.39±0.34	0.023*
Standard devation(SD)			
T-test			
Degree of freedom(Df)			

***P value significant≤ 0.05**

This study discuss the role of bacterial UTI with lactoferrin antibodies in RA disease Most studies agree with UTI can cause systemic autoimmunity and there is experimental and clinical evidence supports the pivotal role of infections in the induction or exacerbation of systemic autoimmunity [5]. Infections can be responsible for aberrant immune response leading to a loss of tolerance towards native proteins, and molecular mimicry between self-antigens and infectious agents is involved in the aberrant immune response [8,9].

Urinary tract is one of the routes that is involved as a site of infection in a substantial number of human beings, especially females, and can be regarded as a route that introduces the infectious pathogen to the immune system; therefore, UTI is expected to be involved in systemic autoimmunity, but the clinical subgroups may react differently with the introduced pathogen or its epitopes [10].

This study represent that the predominant bacteria cause UTI in RA patients is *Proteus spp*. represent as 58.90 % therefore, the principal infection in RA patients might be *Proteus* spp. this result agree with various microbiological and immunological data results support the suggestion that there is a link between RA and UTI mainly caused by *Proteus spp*. [10]. Molecular mimicry and/or cross-reactivity mechanism between Proteus antigens and synovial tissue epitopes (mainly HLA-class II molecules) has been suggested to explain the increased incidence of UTI caused by Proteus and joint destruction in RA [11].

The latter group of investigators has recently confirmed their suggestion by evidence based on observations made in a laboratory animal (rabbit), in which they injected HLA-DR4+ lymphocytes and found that the rabbits were able to produce antibodies that can react with *Proteus spp*., bacteria *in vitro*; moreover, these antibodies were also detected in sera of RA patients [12].

Accordingly and based upon the results from various studies (including the present study),it could be possible to conclude that an evidence exists linking *Proteus* to RA, starting with recurrent sub-clinical *Proteus* UTI and ending in the full development of RA.The cause of this disease is generally agreed to be due to a combined action of genetic and environmental (mainly microbial) factors [2].

The importances of bacterial infection in systemic immunity, RA patients have greater risk to develop different infections, including urinary tract infections (UTI) [10].

Rheumatoid arthritis (RA) is anautoimmune disorder that is characterized by the production of auto antibodies against a variety of antigens. It is possible that more than one process could contribute for different diseases manifestations. One of these may be vascular injury [13].

In addition mean concentraton of lactoferrin Abs in rheumatoid patients with urinary tract infection was significantly higher than that for RA without urinary tract infection and in health controls these results agree with results of a study done by Nässberger. *et al*. [14],while disagrees with a study done by Chikazawa *et al*. [15] **,** that showed no significant differences in lactoferrin antibody levels between RA and healthy controls.

Mean LF Abs in RA with UTI was significantly higher than healthy control (P value ≤ 0.05) agree with Caccavo *et al* [4] and Chikazawa *et al* [15] that found significant correlation between lactoferrin Abs in RA with UTI in comparison to healthy controls.

The presence of these antibodies is simply a reflection of B cell hyperactivity in that anti-LF antibodies bind to LF and block the anti-inflammatory activity of LF, which implies deterioration in host defense against microorganisms and inflammation [16].

UTI may represent important clinical, complications of rheumatoid arthritis, especially if they are investigated in the presence of anti lactoferrin antibodies, so, LF antibodies may considered as a sensitive marker for the diagnosis of UTI caused by inflammatory pathogens in patients with RA and this provides a useful tool for the simple and rapid diagnosis of UTI.

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