



ORIGINAL ARTICLE

ASYMPTOMATIC URINARY TRACT INFECTION BY *PROTEUS MIRABILIS* IN RHEUMATOID ARTHRITIS PATIENTS

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ABSTRACT

Background: *Proteus mirabilis* is thought to contribute in rheumatoid arthritis (RA) development in susceptible individuals through molecular mimicry mechanism. This study was detecting the prevalence of asymptomatic bacteriuria (ABU) caused by *Proteus mirabilis* in RA patients to shed light on its role in RA pathogenesis.

Methods: This work has been conducted in medical Microbiology and Immunology Department and Rheumatology and Rehabilitation Department, Faculty of Medicine, Zagazig University, during the period from May 2017 to May 2018 over a period of 12 months. This study is a case control included 70 RA patients (66 females and 4 males), with age ranged from 25 to 65 years, and 70 healthy controls (67 females and 3 males), with age ranged from 24 to 65 years. Two consecutive urine samples one week interval were collected from each participant for urine count and culture. Blood samples were collected from each participant for detection the level of IgG antibodies against both *Proteus mirabilis* and *E. coli* (the most frequent isolated organism from the RA urine) by homely prepared ELISA.

Results: The ABU was detected in (40%) of RA patients and in (4.3%) of healthy controls. The most common isolated organisms were *E. coli* (50%) followed by *Proteus mirabilis* (25%). A significant difference between both studied groups regarding IgG antibodies levels against *Proteus mirabilis* was detected ($P < 0.001$). No significant difference was observed between both studied groups regarding IgG antibodies levels against *E. coli* ($P = 0.902$). Significant positive correlation was found between *Proteus mirabilis* IgG antibodies levels and the levels of ESR and CRP in RA patients.

In conclusion: *Proteus mirabilis* seems to have a role in RA development.

Keywords: Rheumatoid arthritis, *Proteus mirabilis*, asymptomatic bacteriuria, cross reactivity.

INTRODUCTION

The exact etiology of RA is not clear but medical evidences suggest that it develops frequently in individuals with inherited genetic risk factors after their

exposure to environmental triggers. There are many environmental risk factors, including exposure to infections, hormones, smoking and dietary factors [1].

Numerous studies have shown the clinical association between infection and RA. Infection is often detected in RA early stage and can precede the occurrence of clinical arthritis which suggests that infection contributes to the initiation and exaggeration of RA[2,3].

Proteus mirabilis is thought to contribute in RA development in susceptible individuals through molecular mimicry mechanism. Specifically, molecular similarities have been observed between the ESRRAL amino acid sequence, which is presented in *P. mirabilis* haemolysin and the EQRRAA sequence which is presented in the shared epitope of the RA-linked HLADR molecules. Also molecular similarity has been observed between IRRET amino acid sequence which is presented in *P. mirabilis* urease enzyme and the LRREI amino acid sequence which is presented in type XI collagen [4].

Consequently, repeated infections with *P. mirabilis* will result in the production of high titers of bacterial antibodies. The binding of these antibodies to the cross reactive self antigens will trigger a cascade of inflammatory responses which leads to the production of various chemokines and cytokines leading to a local inflammation with or without systemic effects. In addition, such activation may have a bystander effect on other immune cells, including T lymphocytes, which might result in further enhancement of the inflammatory immune responses with the development of arthritis and the systemic features of this disease[5,6].

METHODS

Study design

A case control study included 70 RA patients and 70 healthy controls was carried out during the period from May 2017 to May 2018 over a period of 12 months. This study was performed in Medical Microbiology & Immunology and Rheumatology & Rehabilitation Departments, Faculty of Medicine, Zagazig University Hospitals. RA patients were diagnosed according to 2010 ACR/EULAR RA classification criteria[7].

Sample size: assuming that percentage of *Proteus mirabilis* in rheumatoid arthritis patients and in control groups is

(48%vs14%)[8]. (respectively so sample size is calculated by Epi Info6 to be 140 cases (70 cases in each group) with confidence level 95% and power of test 80%.

Ethical consideration: All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000 [9]. Institutional Review Board (IRB) of the Faculty of Medicine, Zagazig University approved the study protocol. An informed consent was obtained from all participants of this study and they were told about the aim of the study, and were informed that the data would be used for scientific purposes only.

The following laboratory procedures were performed for the collected specimens including; urine and blood:

Urine samples processing: Two consecutive urine samples "one week interval" were collected from each participant and examined for urine count and bacterial culture. (ABU is defined as a mid-stream urine showing growth of uropathogenic bacteria $\geq 10^5$ CFU/ml in two consecutive samples in women and in a single sample in men[10]. Urine count was done on CLED agar, a count of 10^5 or more was considered significant. All the isolated organisms were identified by gram staining and biochemical reactions.

Homely prepared ELISA: for detection of *Proteus mirabilis* IgG and *E. coli* antibodies in the serum of the study subjects.

- **Preparation of the antigen:** The preserved cultures of *Proteus mirabilis* on brain heart infusion (BHI) agar slants were sub cultured in BHI broth. After 24 hours incubation, the cultures were inoculated onto freshly prepared plates of BHI agar containing 1:800 phenol and incubated 24 hours, the growth on the plates was scraped gently with a sterile loop, suspended in 3 ml sterile normal saline (0.9% w/v). Then the organism was heat killed by maintaining the culture suspension in a heater at 60°C for 30 minutes. The culture suspension was then centrifuged at 3000 RPM for 15 minutes. The supernatant was discarded and the pellet was washed with 400 µl of PBS-T for 3 times.

After the third wash, carbonate-bicarbonate buffer was added to the pellet slowly, and the turbidity was adjusted to 4McFarland turbidity standard tubes.

Coating the ELISA plates with the tested microbial antigens: 100µl of the prepared antigen suspension was added to each well of ELISA plate. The plate was covered with aluminum foil and incubated at 37°C for 3 hours for sensitization. The sensitized plate was then kept at 4°C over night. Each well was aspirated and washed; the process was repeated three times with wash buffer (400 µl of PBS –T). Blocking was done with 150 µl of 1% Bovine serum albumin solution and incubating the plate at 37° C for 2 hours. The plate was again washed 3 times.

ELISA steps: Serum samples were diluted 1:10 using PBS then added to the corresponding wells in duplicate and incubated at 37°C for 90 minutes. The wells were washed 3 times with PBS-T fluid. 100 µl of the Horse Radish Peroxidase conjugated with anti-human IgG was added to each well. The wells were incubated at 37°C for 90 minutes and washed 3 times. The Chromogenic substrate (100 µl) was added. The plate was incubated at 37°C for 20 minutes away from the light. 50 µl of the stop solution was added to each well. Within 30 minutes, the optical density (OD) of each well was determined using ELISA reader (Stat fax 303 plus) set to 492 nm. The mean OD of each duplicate was calculated.

Statistical analysis

The collected data were computerized and statistically analyzed using SPSS program (Statistical Package for Social)

RESULTS

This study has been conducted on two groups, the case group which includes 70 RA patients and the control group which includes 70

apparently healthy volunteers during the period from May 2017 to May 2018.

Science) version 20 (11). Qualitative data were represented as frequencies and relative percentages. Categorical data were compared using Chi-square test.

Table (1) demonstrated that twenty eight out of 70 investigated case group (40%) have ABU in comparison to 3 out of 70 investigated control group (4.3%). There is highly statistically significant difference between both studied groups regarding the frequency of ABU ($P < 0.001$).

Table (2) demonstrated that in the case group (50%) of isolated organisms were *E. coli*, (25%) were *Proteus mirabilis*, (14.3%) were *Enterococci*, and (10.7%) were *Staph saprophyticus*, while in the control group (66.7%) were *E. coli* and (33.3%) were *Staph saprophyticus*.

Table (3) demonstrated that there is highly statistically significant difference between both studied groups regarding *Proteus mirabilis* IgG antibodies levels ($P < 0.001$).

Table (4) demonstrated that there is no statistically significant difference between both studied groups regarding *E. coli* IgG antibodies levels, although the level of *E. coli* IgG antibodies among the case group (0.55 ± 0.13) is higher than this of the control group (0.53 ± 0.06) ($P \leq 0.902$).

Table (5) demonstrated that there is a statistically significant difference between RA patients regarding *Proteus mirabilis* IgG antibodies levels. The highest level of *Proteus mirabilis* IgG antibody was detected in RA patients with *Proteus mirabilis* ABU ($P < 0.045$).

Table (6) demonstrated that there is statistically significant positive correlation between levels of *Proteus mirabilis* IgG antibodies and the levels of ESR and CRP.

Table (1): Frequency distribution of ABU among studied groups

Groups	Case group (70)		Control group (70)		Test	P
	N	%	N	%		
Significant bacteriuria	28	40	3	4.3	Fisher	<0.001**
Non-significant Bacteriuria	42	60	67	95.7		

Table (2): ABU causative organisms in studied groups:

Isolated organisms	Case group (NPI=28)		Control group (NPI=3)	
	N	%	N	%
<i>E. coli</i>	14	50	2	66.7
<i>Proteus mirabilis</i>	7	25	0	0
<i>Enterococci</i>	4	14.3	0	0
<i>Staph. Saprophyticus</i>	3	10.7	1	33.3

Table (3): *P. mirabilis* IgG antibodies among studied groups.

Variable	Case group (N =70)	Control group (N =70)	t test	P
<i>Proteus mirabilis</i> IgG: Mean OD ± SD Range	1.35 ± 0.31 0.654 – 1.76	0.41 ± 0.08 0.301 – 0.567	10.214	<0.001**

Table (4): *E. coli* IgG antibodies among studied groups:

Variable	Case group (N =70)	Control group (N=70)	t test	P
<i>E.coli</i> IgG: Mean OD ± SD Range	0.55 ± 0.13 0.324 – 0.798	0.53 ± 0.06 0.322 – 0.687	0.123	0.902

Table (5): Relation between *Proteus mirabilis* ABU and levels of *Proteus mirabilis* IgG antibodies in the case group:

Case group	<i>Proteus mirabilis</i> IgG Antibodies Mean OD \pm SD	t test	P
Patients with <i>Proteus mirabilis</i> ABU (N=7)	1.62 \pm 0.14	1.959	0.045*
Patients without <i>Proteus mirabilis</i> ABU (N=63)	1.23 \pm 0.3		

Table (6): Correlation between *Proteus mirabilis* IgG antibodies levels, RA duration and the acute phase reactants in in the case group:

Variable	<i>Proteus mirabilis</i> IgG antibodies levels	
	R	P
ESR (mm/hr)	0.906	<0.001**
CRP (mg/dl)	0.847	<0.001**

DISCUSSION

There are numerous ways in which infection can lead to autoimmune diseases, including the initiation of tissue damage leading to the exposure of previously hidden self-antigens or the elaboration of super antigens. Also it has been proposed that similarities in antigenic proteins between infecting pathogens and host tissues may result in immune response against the host tissues [12]. As regards *P. mirabilis* various genetic, microbiological, molecular and immunological studies carried out by independent research groups support its role in RA pathogenesis through recurrent subclinical Urinary tract infection[13].

A case control study was designed and the study participants were classified into 2 groups: a case group which included seventy RA patients (66 females and 4 males), with age ranged from 25 to 65 years and a control group which included seventy apparently healthy volunteers (67 females and 3 males), with age ranged from 24 to 65 years, the two groups were almost matched in sex and age. Our results showed that there was a highly statistically significant difference between both studied groups regarding ABU frequency. It was detected in (40%) of the urine samples from RA patients in comparison to (4.3%) in the healthy control. In our study, *E. coli* was the most common predominant organisms (50%) in RA patients and (66.7%) in the healthy control. *P.*

mirabilis was the second most common isolated organism after *E. coli*. It was isolated from (25%) of the urine samples of RA patients while it was not isolated from the control group. Our result was nearly in agreement with Senior et al who reported slightly elevated rate for isolation of *P. mirabilis* (33%) from the urine of 76 RA patients in comparison to (4%) from the urine of 48 gender-matched healthy individuals[13]. On the contrary, Georgiadou et al did not find any significant differences between RA patients and healthy control regarding ABU frequency[14]. On studying the association between *P. mirabilis* IgG antibodies and RA by Home made ELISA, we used crude *P. mirabilis* antigen preparation. We recorded a highly significant difference between both study groups. The mean OD in RA was (1.35 \pm 0.31) versus (0.41 \pm 0.08) in the control group. Also Raj Kumar et al reported that the mean antibody titre against *P. mirabilis* in RA was (1.74 \pm 0.6) and this was significantly higher than in the control group (0.13 \pm 0.3) [8].

On the other hand, Christopoulos et al used three synthetic amino acid peptides. Each one of them contained a homologous amino acid sequence with *P. mirabilis* enzyme [12]. They found that RA patients presented significant elevated levels of antibodies against these synthetic peptides when compared to healthy controls. Based on these results, both home

made ELISA using crude antigens and synthetic peptides reported significant increase in the levels of *P. mirabilis* IgG antibodies in RA patients in comparison to the healthy controls. However, the use of these synthetic amino acid peptides could help in identification the exact cross-reactive epitopes between human tissue antigens and *P. mirabilis*. Production of vaccine lacking these cross-reactive epitopes will be possible. This approach may be effective for susceptible individuals to resist RA development. However, the use of these synthetic amino acid peptides was expensive and not available in our study so we use crude antigen preparation. In the present study, *E. coli* was the most frequent isolated organism from RA patients(50%), so it was indicated to evaluate its association with RA (if any) in comparison to *Proteus mirabilis*. We found no significant differences between both study groups regarding *E. coli* IgG antibodies.

In the current study, the highest levels of of *P. mirabilis* IgG antibodies were detected in patients with *P. mirabilis* ABU. The mean OD± SD of these antibodies was (1.62±0.14) versus (1.23±0.3) in other RA patients.

Our results showed a significant positive correlation between *P. mirabilis* IgG antibodies levels and the levels of the ESR and CRP in RA patients. It is in agreement with Rashid and Ebringer who reported the same results (15). The elevation of ESR and CRP levels is usually secondary to the result of immunological binding between *P. mirabilis* antibodies presenting in high titres and the cross-reactive HLA epitopes in synovial tissues, which leads to the activation of complements and other inflammatory cascades.

Limitation of this study: already synthesized ELISA was not available so we performed homely prepared ELISA. Also synthetic peptides were not available so we used crude antigens.

CONCLUSION

Proteus mirabilis seems to have a role in RA development. So we recommend carrying further studies using synthetic peptides for detection of the cross reactive epitopes between *Proteus mirabilis* and human tissue antigens which will help in production of

Proteus mirabilis vaccine lacking its cross reactive epitopes with human tissue antigens as a new strategy for RA eradication.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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