Original Article

Antiphospholipid Antibodies in Women with Recurrent Urinary Tract Infection

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Abstract

Background: A variety of infections, including acute and recurrent urinary tract infections (UTIs), can trigger production of antiphospholipid antibodies (aPL). These antibodies in women can lead to recurrent pregnancy loss. The aim of this study was to evaluate the prevalence of aPL in recurrent UTI patients.**Materials and Methods:** A total 52 subjects who had positive urine culture and 50 healthy individuals as controls were evaluated for presence of lupus anticoagulant (LA), anticardiolipin, anti-β2 GPI(anti-β2-glycoprotein I) autoantibodies IgM and IgG and Interleukin-8 levels. Determination of lupus anticoagulant was done by Activated Partial Thromboplastin Time (aPTT). Anticardiolipin and anti-β2 GPI autoantibodies were evaluated by ELISA method. Interleukin-8 values were also evaluated using ELISA method. **Results:** Escherichia coli (86.61%) and Proteus mirabilis (1.92%) had the highest and lowest frequency respectively. The prevalence of anti- β2 GPI IgG and IgM isotypes and anticardiolipin IgG and IgM isotypes or LA in UTI patients and healthy controls was 0.0%. There was significant association between neutrophil counts and IL-8 levels at the $p < 0.01$.**Conclusion:** Our results showed that in the UTI group and controls evaluated antiphospholipid antibodies were not present. The production of antiphospholipid antibodies is influenced by various genetic and environmental factors and chronic urinary tract infection alone is not the cause. This can affect the prevalence of antiphospholipid antibodies in various populations. However, other factors, such as the type of antiphospholipid antibody, sampling season and methodology can affect the results.

Keywords: Antiphospholipid antibodies, Recurrent urinary tract infections, Recurrent pregnancy loss

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Introduction

A combination of host genetic factors and exposure to environmental factors triggers the development of autoimmune diseases (1). Microbial agents or viruses, as environmental factors can induce autoimmune diseases by a variety of mechanisms such as polyclonal activation, unbalancing the immune response, their superantigens, release of cytokines and chemokines and molecular mimicry (1, 2).

In the case of antiphospholipid syndrome (APS), anticardiolipin and anti-β2 glycoprotein I pathogenic antibodies are detected. Although there is molecular mimicry between β2 GPI and infections, such as cytomegalovirus, haemophilus influenza, neisseria gonorrhoeae, rubella, toxoplasma and tetanus toxoid, and IgM antibodies against them have been detected, the direct connection between these infections and APS has not been established (1). The classical antiphospholipid syndrome is characterized by the presence of antiphospholipid antibodies (aPL) which bind target phospholipid molecules, mainly through β2 GPI, and are associated with recurrent fetal

loss and thromboembolic phenomena. Many infections are associated with increases in aPL. Skin infections (18%), human immunodeficiency virus (HIV) infection (17%), pneumonia (14%), hepatitis C virus (HCV) (13%), and urinary tract infections (10%) constituted the most common infections found as ‘‘triggering’’ factors (2,3).

Urinary tract infections (UTI) are one of the most common infections in women worldwide and Escherichia coli is the most prevalent causative organism of UTI and is solely responsible for more than 80% of these infections (4,5). Recent years, the understanding of the host-pathogen interaction and activation of the immune response in the urinary tract has increased considerably. Attachment to the uroepithelial cells by bacterial fimbriae allows for close contact between host and pathogen. Transmembrane signaling through TLRs leads to the production of inflammatory mediators such as chemokines with subsequent recruitment of professional immune cells to the infectious focus. Specifically, the CXC chemokine IL-8 is needed for neutrophil recruitment and activation in the urinary tract. The neutrophils may however also, cause unwanted effects for the host such as tissue destruction and autoantibody production (6).

Because women with recurrent urinary tract infection may produce aPL and are prone to recurrent miscarriage, the aim of this study was to evaluate the prevalence of aPL in recurrent UTI patients.

Methods

Patient selection. A total 52 subjects who had positive urine culture and 50 healthy individuals as controls were selected from the patients referred to Bu- Ali Polyclinic of Qom, Iran and enrolled in this study (Summer et al, 2015). Informed consent was obtained from all participants and approved by the ethical committee of the Qom University of Medical Sciences. Questionnaire consisting of a series of questions about age, history of infection, drug medication, miscarriages, and history of bruising, burning sensation, stroke, cardiovascular diseases, pregnancy, diabetes, systemic lupus erythematosus (SLE) and other autoimmune diseases were completed by all participants. Also participants were interviewed and examined by physician to determine characteristics and medical conditions. The complete history was taken and clinical assessment was done. All patients were nonsmokers, had no history of medical situations such as, chronic viral infections (e.g. HBV, HCV and HIV), Helicobacter pylori infection, Neisseria gonorrhoeae infection, recent non chronic infections (e.g. Streptococcal infections), diabetes mellitus, aspirin, heparin, warfarin or corticosteroid intake; chronic systemic disease including any autoimmune diseases, car¬diopulmonary diseases; history of surgery, chronic renal failure (creatinine clearance test <30 ml/min), a chronic liver diseases, connective tissue diseases, permanent urinary catheter, urinary tract stent, nephrostomy tube, urinary incontinence, neurogenic bladder, kidney malformations, kidney stones, gynecological problems, malignancy and pregnancy. So, patients did not have any problems except for urinary tract infections.

Urine culture, bacterial colony count and identify types of bacteria. Patients with clinical symptoms of UTI referred to Bu- Ali Polyclinic of Qom, Iran, were investigated. Clean-Catch midstream urine of the patients was collected in a sterile tube (4-5 ml) and immediately transported to the laboratory. Guidelines for proper specimen collection were given to all patients on a printed card (7). A measured amount of urine, using calibrated loop method was inoculated to nutrient agar medium (Merck, Germany) for colony count. Equal or more than 104 CFU/ml of a single potential pathogen interpreted as positive UTI and a result of 102-104 CFU/ml was repeated. A less than102 CFU/ml was interpreted as negative UTI (8). Urine specimens were cultured for isolation of the microbial agents of UTI on blood agar and Mac Conky agar media (Himedia, India & Merck, Germany). All the bacteria isolated from urine in this study were identified using conventional biochemical tests (7, 9, 10).

The criteria for recurrent urinary tract infection were three UTIs with three positive urine cultures during a 12-month period, or two infections during the previous 6 months (11-14).

Autoantibodies evaluation

Determination of lupus anticoagulant. We used a lupus-sensitive activated partial thromboplastin time. Standard aPTT was performed by incubating 0.1

ml of patients or controls plasma with 0.1 ml of aPTT (Thermo Fisher Scientific, USA) for 5 min at 37°C, after which 0.1 ml of 25 mM CaCl2 was added and the clotting time recorded. Samples with aPTT values above the 35.2 seconds were regarded as positive.

Determination of anticardiolipin , anti-β2 GPI autoantibodies IgM and IgG. Serum levels of anticardiolipin IgM and IgG and anti-β2 GPI IgG and IgM levels antibodies were measured using EUROIMMUN Medizinsche Labordiagnostika GA (Germany). According to the manufacturer's instructions, the cut-off values for positivity were ≥12 IgG or IgM for anticardiolipin. Specimens with less than 12 PL-IgM-u/ml or12 PL-IgG-u/ml were considered negative. For anti-β2 GPI IgG and IgM the cut-off values were ≥20 RU/ml. Specimens with less than 20 RU/ml were considered negative.

Interleukin-8 assessment. Serum quantification of IL-8 was performed with ELISA using affimetrix eBiosciences, Austria, with a detection limit of 2.0 pg/mL.

WBC and platelet count. WBC and platelet count was performed by BC-5800 hematology analyzer (Mindray, China).

CRP assessment. Serum CRP was measured by immunoturbidimetric method (COBAS, Roche Diagnostics, Basel, Switzerland).

Statistical analysis. Data and statistical analysis was done using SPSS 16 software. The data were expressed as mean ± SD and proportions were expressed as percentage. Normality distribu¬tion of data was first determined by Kolmogorov–Smirnov test. Variables were compared with the independent t-test or Mann–Whitney U-test. Correlations were calculated by Pearson or Spearman. P-value of less than 0.05 was considered statistically significant for all tests.

Results

Minimum and maximum age of the UTI patients was 12 and 51 years respectively. All Patients had a history of 7 to 9 UTI in the last three years. The frequency and percentage of bacteria isolated from UTI patients are presented in Table1. Escherichia coli (86.61%) and Proteus mirabilis (1.92%) had the highest and lowest frequency respectively.

Table1. The frequency and percentage of bacteria isolated from UTI patients.

	Frequency	%
Escherichia coli	44	86.61
Staphylococcus saprophyticus	3	5.77
Klebsiella pneumoniae	2	3.85
Enterobacter gergoviae	2	3.85
Proteus mirabilis	1	1.92
Total	52	100

The prevalence of anti- β2 GP1 IgG and IgM isotypes and anticardiolipin IgG and IgM isotypes or LA in UTI patients and healthy controls was 0.0% (Table 2).

Table2. Demographic and immunological characteristics of the study and control groups

Characteristics	Patients(n=52)	Controls(n=50)	P value
Age(years)	27.87±9.37	27.94±9.18	0.97
Anti- β2 GP1 IgG	0(0.0)	0(0.0)	
Anti- β2 GP1 IgM	0(0.0)	0(0.0)	
LA(%)	0(0.0)	0(0.0)	
aCL- IgM(%)	0(0.0)	0(0.0)	
aCL- IgG(%)	0(0.0)	0(0.0)	
WBC(cells/mm3)	7421.15±1897.09	6344.00±1466.10	0.004
IL-8(Pg/ml)	82.98±185.15	18.05±13.13	0.027
Plt(/mm3)	210.11±48.70	208.44±54.85	0.89
CRP(mg/L)	44.22±12.14	14.05±12.01	0.027

There was significant association between WBC and IL-8 levels at the $p < 0.01$. There was no significant association between age and IL-8 levels in both patients and control groups. Thrombocytopenia(less than 150,000 platelets per microliter) was observed in 6 patients. None of the controls had thrombocytopenia.

Discussion

Our results showed that in the UTI group and controls, the anti- β2 GPI IgG and IgM isotypes and anticardiolipin IgG and IgM isotypes or LA were not present. But, the increase in leukocytes numbers, CRP and IL-8 concentrations were observed in patients. Therefore, it can be assumed that infections alone cannot trigger production of pathogenic aPL antibodies.

However, it was postulated that infections may be a trigger factor for the induction of pathogenic aPL in certain predisposed individuals. In fact aPLs have been found not only in patients with autoimmune diseases like SLE, but also in patients with various infections (15, 16). Asherson and et al. reported the presence of antiphospholipid antibodies in infectious diseases such as AIDS, tuberculosis, measles, chickenpox, hepatitis and others. They reported that 35% of catastrophic APS cases were preceded by an infection, with urinary tract infections reported to occur in 6% of the cases (17). These data suggests that urinary tract infections in a small percentage of patients may cause aPL production and other infections may be more important.

In the general population, 1% to 5% of people have positive aPL (18). Infection-associated aPL appear temporarily and disappear within 2 or 3 months in most cases (19).

It has recently been demonstrated that there is a seasonal influence on the prevalence of these antibodies in normal healthy populations, with a higher prevalence in the winter months than in the summer; the significance of these findings with respect to the etiology of this disorder and to thromboembolism is not yet known. Familial clustering of raised aPL antibody levels and HLA linkages indicate that the antibodies probably occur in genetically susceptible hosts in response to some antigenic challenge (20). Since sampling was conducted in the summer, our results can be attributed to it.

One of the clues linking cell death to the onset of autoimmunity is provided by autoantibodies that bind apoptotic cells and recognize surface epitopes that include complexes of anionic phospholipids, such as phosphatidylserine (PS) and β2 GPI(21, 22). Presumably, infection may cause inflammation and is not initial factor for production of autoantibody in infected individuals. So, appropriate removal of the apoptotic cells prevents the production of pathogenic autoantibodies.

Other potential environmental triggers of APS development include vaccination, drugs and certain malignancies. However, to date, there is no conclusive evidence linking vaccination to the development of APS (23, 24). In this study, patients and controls were not taking other drugs (such as: chlorpromazine, amoxicillin, phenytoin, chlorothiazide, propranolol, antiarrhythmic drugs, antihypertensive medications, quinine, alpha-interferon, or Infliximab) except antibiotics and oral contraceptives. This may be one of the reasons for our results.

Various animal models and family and population studies have been used to highlight HLA associations with the occurrence of aPL and the development of thrombosis in aPL-positive patients. Thus, Major Histocompatibility Complex (MHC) genes may influence not only autoantibody production but also disease expression itself (25). Other genes outside the MHC region also contribute to both autoantibody production and disease expression in APS. A polymorphism in domain 5 of β2 GPI, valine instead of leucine at position 247, is found more frequently in patients with APS than matched controls and is associated with anti β 2GPI autoantibody production in susceptible patients (26-28). The study did not examine patients genetically and maybe our cases were not genetically predisposed to produce antiphosphplipid antibodies.

The clinical importance of IgA anti-β2 GPI has increased in the last few years due to the utilization of kits useful to detect IgA anti- β2 GPI and the task

force in the 13th International Congress on Antiphospholipid Antibodies (2010, Galveston, TX, USA) recommended testing for the IgA anti-β2 GPI in cases negative for IgG and IgM and when APS is still suspected (29). In this study, IgA anti-β2 GPI was not measured.

It has been proposed that aPL belong to the natural antibodies, because they share many properties with these B1 cell derived antibodies. A non-specific stimulus by pathogen associated patterns (PAMP) which can activate pattern recognition receptors, e.g., toll-like receptors (TLR) stimulates an increase over basal antibody production by B1 cells. Subsequently, antigen producing B1 cell clones are positively selected by exposure to their autoantigens (30). Natural antibodies are those normally present and are not masked if the antigen is not normally present, as in the case of ABO blood group antibodies and others, e.g. If the antigen is normally present, the natural autoantibody is suppressed, either by circulating as an immune complex or by antiidiotype antibodies (31).

Another factor that influences results can be the method. It is well recognized that conflicting reports are common in the literature on aPL detected by ELISA methods. Methodological pitfalls in aPL testing by ELISA techniques (e.g. Plate plastic, source and handling of aPL, blocking, washing, dilution, heat inactivation, temperature, calcium, serum vs plasma) may affect our results (31).

It seems that neutrophilia and high levels of interleukin-8 and CRP represents innate immune activation, and the production of pathologic antibodies requires the activation of acquired immunity and T cells (32).

The most likely scenario is a complex interplay of a multitude of environmental factors in a genetically susceptible patient, which then induces autoantibody development and consequently typical disease manifestations (32). Another hypothesis is that the prevalence of antiphospholipid antibodies is low among our patients truly.

Conclusion

In summary, various factors contribute to the development and detection of pathological autoantibodies, and apparently chronic UTI alone is not sufficient to stimulate pathologic antibodies production. In the other hand, it seems that a number of positive cases have been eliminated due to the inappropriate methodology.

It is recommended that antiphospholipid IgA class, two types of different ELISA kits and lastly, other antiphospholipid antibodies are examined.

Conflicts of Interest

The authors declare that there is no conflict of interest.

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