RESEARCH ARTICLE

Investigation Few Biomarkers for Pantoea Infection with Systemic Lupus Erythematosus Patients

Burhan T Burhan1*, Layla S A Hassan2

1Department of Medical Laboratory Techniques, College of Health and Medical Techniques, Kufa, Al-Furat Al-Awsat Technical University, Al-Kufa, Iraq.

2Department of Community Health Techniques, College of Health and Medical Techniques, Kufa, Al-Furat Al-Awsat Technical University, Al-Kufa, Iraq.

Received: 12th November, 2023; Revised: 12th December, 2023; Accepted: 19th December, 2023; Available Online: 25th March, 2024

ABSTRACT

Background: Systemic lupus erythematosus (SLE) is a disease known as systemic lupus erythematosus. SLE is an autoimmune condition in which the immune system attacks the body’s own tissues, leading to extensive tissue damage and inflammation in other organs. One of the most frequent causes of death and morbidity in SLE patients is infection. Patients with SLE may contract a genus of gram-negative bacteria called Pantoea. This study aims to find biomarkers for Pantoea infection in SLE patients in Iraq’s Najaf Governorate.

Methods: About 60 individuals with systemic lupus erythematosus from Al-Sader Medical City in Al-Najaf Province had blood samples taken at a specialized rheumatology and nephrology center between September 2022 and February 2023. Some bio-markers has been evaluated.

Results: The results of microbiological analyses indicate that 26/60 samples contain bacteria. Pantoea was discovered in 3 (11.6%) of the 26 specimens. Despite SLE, increased autoantibody levels in patients. The results of this analysis indicate a significant decrease in Hb, WBC, and platelet levels. In contrast to the control group, the serum levels of CD69, IL-21, and IL-35 increased significantly.

Conclusion: Pantoea produces elevated diagnostic and immunological parameters in SLE patients. Consequently, it is necessary to conduct assays to identify bacterial infections in patients, with a significant increase in CD69, IL-21, and IL-35 in the event that SLE patients are infected with bacteria.

Keywords: Systemic lupus erythematosus, Pantoea, Cluster of differentiation 69, Interleukin 21, Interleukin 35.

International Journal of Drug Delivery Technology (2024); DOI: 10.25258/ijdtt.14.1.08

How to cite this article: Burhan BT, Hassan LSA. Investigation Few Biomarkers for Pantoea Infection with Systemic Lupus Erythematosus Patients. International Journal of Drug Delivery Technology. 2024;14(1):45-49.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

The usual systemic autoimmune illness is systemic lupus erythematosus (SLE). It is distinguished by a range of clinical symptoms, varying degrees of severity, and alternating stages of remission and aggravation.1 Arthritis, rashes, serositis, multiple cytopenias, renal disease, and behavioral and neurological problems, among other signs, can occur in SLE patients.2 A multisystemic illness can be predicted by the presence of particular autoantibodies years before it shows clinical symptoms.3 Numerous autoantibodies and clusters of autoantibodies have been linked to the illness’s incidence and its particular symptoms.4 Infectious illness is one of the main causes of morbidity and death in people with SLE. Major infections are responsible for a significant amount of hospitalizations and deaths, occurring in at least one in 29% of patients. Microorganisms are responsible for around 80% of infections associated with SLE.5 It was shown that bacteria caused 78.6% of all infections in people with SLE. This data is in line with other studies showing that SLE patients from India, Malaysia, Singapore, Spain, and the United States might have bacterial infections up to 80% of the time.6 Gram-negative, rod-shaped, facultatively anaerobic Enterobacteriaceae are Pantoea spp. Peritrichous flagella are what move it forward.7 Its previous names were Erwina and Enterobacter Pantoea spp., and as a plant pathogen, it is often found in environmental habitats such soil and water, vegetables, and seeds.8 Research in recent years has revealed that Pantoea species are becoming more and more responsible for human illnesses.9 Numerous units, including the intensive care, burn, hemodialysis, and cancer departments, have reported a considerable rise in

*Author for Correspondence: burhansaadradhy12@gmail.com
nousomial infections. This has caused sepsis to evolve, a complicated, multifaceted phenomenon that can result in illnesses of varied degrees of severity, such as acute sepsis or septic shock. Most clinical indicators are vague, and in certain circumstances, pinpointing the infection’s origin may be challenging or impossible. In the Iraqi province of Najaf, this project attempts to find biomarkers for Pantoea infection in individuals with systemic lupus erythematosus.

MATERIALS AND METHODS
From September 2022 to February 2023, blood samples from 60 patients with systemic lupus erythematosus ages 16 to 56 were gathered. Al-Sader Medical City in the Province of Al-Najaf, a specialized center for rheumatology and nephrology, provided the samples. First, patients were interviewed using a confidential questionnaire covering their personal information and medical history. All participants gave verbal informed consent, which was in accordance with the center’s code of ethics.

Collect Samples
Ten mL of blood were extracted from each patient and healthy individual (control). One 3 mL sample is transferred to a 6 mL gel tube and at least 30 minutes at room temperature to permit coagulation. After 10 minutes of centrifugation at 3000 rpm, the resulting serum is used to measure immunological parameters. About 1-mL of whole blood is placed in an ethylenediaminetetraacetic acid (EDTA) tube for hematological parameters, and 1-mL is used for the ESR test. About 5 mL of blood specimen was inoculated into bactaluter bottles, which were then incubated at 37°C for 2 to 7 days in a bactaluter automated blood culture. Evaluate ANA and anti-ds-DNA antibodies using The ELISA kits (Aeskulis, Germany) and evaluate cytokines CD69, IL-21 and IL35 using The Elisa kits (Melsen, China), radial immunodiffusion plate kits (LTA, Italy) for complement C3 and C4. Patients with SLE should also provide samples of their urine and skin, which should be on blood agar and MacConkey agar for culture.

Isolation and Identification of Bacteria
MacFaddin approved cultured and microscopic characteristics and biochemical assays were used to identify bacterial isolates. Using the Vitek 2 compact system. The diagnostic was likewise done in line with the manufacturer’s recommendations.

RESULTS
The findings of the investigation as shown in Figure 1, of the 60 clinical samples collected from systemic lupus erythematosus patients, 26 (43.3%) specimens from 22 female and 4 male patients contained bacterial growth, while the remaining 34 (56.70%) specimens exhibited no growth.

The ratio of female to male SLE patients was 19:1, with 57 females (83.30%) and three males (16.70%). The 9:1 ratio consisted of 37 females and three males from the healthy control group. In our study of SLE patients, 23.33% of patients had a family history, compared to 76.60% with no family history. The domicile of SLE patients in our study was found to be 39 (65%) in urban regions compared to 21 (35%) in rural regions, as illustrated in Figure 2.

About 17 gram-negative bacteria that were identified were 4/17 Escherichia coli, 5/17 Klebsiella pneumoniae, 5/17 Salmonella typhi and 3/17 Pantoea. Pantoea was found in 3 specimens one of them was obtained from blood the second was obtained from urine and the last specimen was obtained from skin.

Cultural and Morphological Characteristics
Pantoea sp as depicted in Figure 3, slow ferment lactose colonies on MacConkey agar appeared as medium-sized, smooth, pink colonies with punctuation.

As indicated in Table 1, biochemical tests showed that all isolates varied for various biochemical responses but were positive for catalase, citrate, motility, and indole and H2S generation.

The VITEK 2 system was used as the final confirmatory stage for detecting Pantoea after biochemical testing and final diagnosis.

Immunological Study
The ELISA was used to measure certain types of autoantibodies, and it was found that the level of anti-dsDNA antibody in patients with SLE Pantoea infection was significantly higher (40.06 ± 0.09) than in the control group (7.25 ± 0.09 IU/mL). In addition, it has been determined that the concentration of ANA antibody is highest in patients with SLE (2.2 ± 0.02 IU/mL), compared to healthy humans (0.4 ± 0.08 IU/mL) (Figure 4).

The radial immunodiffusion assay revealed a significant decrease (p ≤ 0.05) in the serum of SLE patients with Pantoea infection (80.34 ± 0.07, 17.63 ± 0.14 mg/dl) compared to healthy individuals (105.5 ± 0.09, 36.2 ± 0.09 mg/dl) when evaluating the level of serum complement C3, C4 in the total for the study.

Cluster of Differentiation 69 (CD69)
The results of the statistical analysis of the study are depicted in Figure 5 as a significant p ≤ 0.05 increase in the level of CD69 in the serum of Pantoea-infected patients compared to healthy serum levels. The serum CD69 levels were 24.34 ± 30.05 ng/mL compared to 10.96 ± 51.16 ng/mL in healthy serum.
Investigation Few Biomarkers for *Pantoea* Infection

The results indicate a statistically significant increase (p ≤ 0.05) in the serum levels of IL-21 and IL-35 in *Pantoea*-infected SLE patients 22.523 ± 0.01 and 18.465 ± 0.01 compared to healthy serum 8.07 ± 32.9 and 12.14 ± 20.13, as illustrated in Figure 6.

**Table 1:** Biochemical test for *Pantoea* species identification

<table>
<thead>
<tr>
<th>Test</th>
<th><em>Pantoea</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram stain</td>
<td>-</td>
</tr>
<tr>
<td>Catalase</td>
<td>+</td>
</tr>
<tr>
<td>Oxidase</td>
<td>-</td>
</tr>
<tr>
<td>Hemolysin</td>
<td>-</td>
</tr>
<tr>
<td>Methyl-red</td>
<td>+</td>
</tr>
<tr>
<td>Vogas-proskauer</td>
<td>+</td>
</tr>
<tr>
<td>Urease</td>
<td>-</td>
</tr>
<tr>
<td>Indole</td>
<td>-</td>
</tr>
<tr>
<td>Citrate utilization</td>
<td>+</td>
</tr>
<tr>
<td>Kligler Iron agar (gas/H2S)</td>
<td>Acid/acid with gas/ H2S -</td>
</tr>
</tbody>
</table>

**DISCUSSION**

SLE is a systemic autoimmune illness that has complicated clinical symptoms and a propensity for excessive B and T cell activation, autoantibody formation, and tissue and organ destruction. Despite improvements in SLE diagnosis and therapy, the disease’s etiology and pathophysiology are still poorly understood. Hence, treatment effectiveness is still insufficient. SLE-related morbidity and death are increasing. Diagnoses are often challenging or delayed, necessitating clinical skill to combine clinical and immunological findings.
Infectious illness is one of the main causes of morbidity and death in people with SLE. Major infections are responsible for a significant amount of hospitalizations and deaths, occurring in at least one in 29% of patients. Microorganisms are responsible for around 80% of infections associated with SLE. The vast family Enterobacteriaceae, of which many species are pathogens or trigger inflammatory responses, is a member of the Proteobacteria phylum. By affecting T cells, changes in the prevalence of Enterobacteriaceae may have an impact on the onset and progression of SLE. One of the most common causes of lung infections is the family Enterobacteriaceae. Therefore, one of the things that contributes to the many lung and stomach infections that SLE patients experience may be the increased number of Enterobacteriaceae in their intestines. *Pantoea agglomerans* is often kept apart from food, water, soil, and plants. This organism is an opportunistic pathogen; infection usually requires an immunocompromised host. Nevertheless, despite the rarity of human infections, they can be brought on by trauma from contact with plant material while performing agricultural tasks, gardening, or watching children play, as well as secondary bacteremia or nosocomial infections brought on by contaminated intravenous fluids or catheters in medical equipment. In addition, *P. agglomerans* is commonly the source of a range of occupational disorders brought on by the allergic and/or immunotoxic effects of the endotoxins and allergens this pathogen produces. In our research, ANA and anti-dsDNA antibodies are markedly higher when compared to healthy controls. Comparatively, to healthy controls, complement levels are considerably lower. A mechanism for how SLE is induced in genetically vulnerable people and how this leads to the breakdown of self-tolerance has been proposed: molecular mimicry between infectious pathogen molecules and SLE-related autoAgs. Notably, both in humans and animals, bacterial infections have been associated with the production of antinuclear antibodies specific for nucleic acids. According to our theory, curli amyloids trigger the formation of anti-dsDNA autoAbs by exposing bacterial DNA to autoreactive B cells and doing so *via* a molecular mimicry mechanism. A range of cells, including immune and non-immune cells, synthesize and produce a class of tiny proteins known as cytokines. Both endogenous and exogenous cytokines maintain the immune system's delicate equilibrium, and when this balance is upset, it may lead to crippling immune-related disorders. IFN-γ production and CD69 expression are both enhanced in NK cells from peripheral blood in rheumatic disorders such as SLE, which is associated with higher serum levels of IFN-α, a crucial cytokine in the pathogenesis of this condition. These individuals also exhibit increased amounts of CD8+ CD69+ cytotoxic cells, which may cause a variety of cell types to undergo apoptosis. Additionally, these individuals exhibit higher than average concentrations of cytotoxic CD8+ CD69+ cells that may cause apoptosis in a variety of cell types. A cytokine called interleukin 21 (IL-21) binds to a receptor made up of the public cytokine receptor γ chain (γc) and the private interleukin 21 receptor (IL21R). In lymphohematopoietic cells, the IL21R is widely distributed, and IL21 affects a variety of cell types, including CD8+ memory T cells, NK cells, and subsets of CD4 memory T cells. A crucial role of IL21 is the encouragement of B-cell activation, differentiation, or death during humoral immune responses. Certain autoimmune illnesses are characterized by increased IL21 production, which is most likely to support the development of autoantibodies and the clinical features of autoimmune disease. Autoimmune disorders include a variety of illnesses distinguished by diverse elements that lead to a breakdown in self-tolerance. The etiology of many autoimmune disorders depends heavily on immunity mediated by cytokines. Recent research have shown that the recently discovered cytokine interleukin-35 (IL-35), a member of the IL-12 family, has a role in the development of autoimmune illnesses such as SLE, rheumatoid arthritis (RA), and systemic sclerosis (SSc).

REFERENCES


Investigation Few Biomarkers for Pantoea Infection


