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# EVALUATION OF SOME IMMUNOLOGICAL PARAMETERS FOR STAPHYLOCOCCUS XYLOSUS INFECTIONS IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

**Burhan T. Burhan[1]*, Layla S. Abdul-Hassan[2]**

[1'2]College of Health and Medical Techniques /Kufa, Al-Furat Al-Awsat Techniques University

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**Corresponding Author:**

Burhan T. Burhan

Email: Burhantahseen116@gmail.com



ABSTRACT

***Purpose**: Systemic lupus erythematosus (SLE) is a chronic, systemic autoimmune illness that manifests clinically as well as immunologically and in several lab tests abnormalities. Patients with SLE frequently get infections, which account for 30–50% of morbidity and death. The bacterial species Staphylococcus xylosus is a member of the Staphylococcus genus. This study's objective was to assess several immunological indicators of S.xylosus infections in individuals with systemic lupus erythematosus in the Najaf Governorate of Iraq.*

***Subjects and Methods:** Blood samples were taken from 60 SLE patients ranging in age from 16 to 56. Each patient has had a sample of 10 milliliters of blood taken. 5 ml were used to measure immunologic parameters, and another 5 ml were used to diagnose S. Xylosus by culturing a sample on blood agar and mannitol salt agar. culture on mannitol salt agar and blood agar.*

***Results:** According to the microbiological tests, 26 specimens (or 43.3%) had isolated bacteria, whereas 34 specimens (or 56.7%) had no sign of bacterial growth. Out of the 26 blood samples collected, S.xylosus was detected in 2 samples. normal amounts of ANA and anti ds-DNA despite SLE sufferers. The results of this study showed that serum levels of CD69, IL-21, and IL-35 significantly increased when compared with controls, despite a substantial drop in Hb, WBC, and platelet counts but an elevated ESR.*

***Conclusions:** xylosus produces normal autoantibody levels that are utilized to diagnose SLE. An important factor in the pathophysiology of SLE, which causes the disease to develop, is a high concentration of inflammatory cytokines.*

## INTRODUCTION

The most common autoimmune disorder is systemic lupus erythematosus (SLE). Faulty apoptotic clearance, stimulation of innate and adaptive immune systems, activation of complement, creation of immune complexes, and tissue inflammation all play a role in the development of a self-perpetuating autoimmune illness. It's believed that a number of pathogenic pathways contribute to SLE clinical symptoms. Although SLE can impact several body systems, the distribution of its clinical symptoms and autoimmune manifestations varies widely across individuals and, in some cases, even evolves over time (Fava & Petri, 2019). Multiple autoantibodies (Ab) that result in the

development and deposition of immune complexes (ICs) as well as other immunological processes are linked to the signs of SLE. The majority of SLE patients report constitutional, mucocutaneous, and musculoskeletal manifestations as their first and most frequent symptoms. The epidermal, hematologic, renal, neuropsychiatric (NP), cardiovascular, and/or respiratory systems can all be impacted, in addition to any organ. Not all manifestations inevitably happen at once, and certain symptoms may not show up for months or even years at a time (Basta *et al*., 2020). Patients with systemic lupus erythematosus (lupus) frequently experience bacterial infections of the lung, skin, blood, and other tissues, which are frequently more severe and invasive than comparable infections in control groups (Battaglia & Garrent-Sinha, 2021). Through the stimulation of the immune system by their by-products (such as lipopolysaccharides or nucleic acids), bacteria have also been linked to the pathogenesis of SLE. In this intricate mechanism, bacteria and bacterial byproducts engage Toll-like receptors, triggering B, T, and antigen-presenting cells, which in turn triggers the release of pro-inflammatory cytokines and antibody formation (Rigante *et al*., 2014). A coagulase-negative staphylococcus is Staphylococcus xylosus. It is a commensal bacteria found in skin and mucous membranes, and on rare occasions, it can lead to illnesses in people (Giordano *et al*., 2016). Very rarely, S.xylosus has been determined to be the source of a human infection; however, in some instances, this conclusion may have been incorrect (Irlinger, 2008). The study's objective is to examine various immunological indicators for S.xylosus infections in SLE patients in the Iraqi province of Najaf.

## METHODOLOGY

Data gathering From September 2022 to February 2023, 60 individuals with Systemic Lupus Erythematosus, ranging in age from 16 to 56, had blood samples taken. samples obtained from the rheumatology and nephrology-focused Al-Sader Medical City in the Al-Najaf Province. An anonymous questionnaire covering personal data and medical history was first used to individually interrogate patients. All subjects verbally consented after being fully informed, which was in keeping with the specialized center's ethical standards.

### Collect blood samples

Blood samples weighing ten milliliters were taken from both the patients and the healthy controls. After being centrifuged at 3000 rpm for 10 minutes, one sample of 3 ml was transferred to a 6 ml gel tube and allowed to clot at room temperature for at least 30 minutes to clot. The serum obtained from this process was used to assess several immunological parameters. For haematological parameters and the ESR test, 1 ml of whole blood is placed in an EDTA tube. Bact/alert bootles were infected with 5 ml of blood samples and incubated for (2– 7) days at 37°C in a bact/alert automated blood culture. All specimens were grown on mannitol salt agar and blood agar. Evaluate cytokines CD69, IL-21, and IL35 by Elisa kits (Melsen, China), ANA and Anti-ds-DNA antibodies by The ELISA kits (Aeskulisa, Germany), and complement C3 and C4 by radial immunodiffusion plate kits (LTA, Italy).

### Isolation and Identification of bacteria

Bacterial isolates were recognized based on cultured, microscopy, and MacFaddin- approved (Mac, 1976) biochemical assays. Additionally, a diagnosis was made utilizing the vitek 2 compact system and in accordance with the guidelines provided by the creator.

## RESULTS AND DISCUSSION

### Isolation and identification

In (60) clinical samples taken from patients with systemic lupus erythematosus, the results of microbiological investigations revealed 26 specimens with bacterial infection, 22 female and 4 male specimens with isolated bacteria, and 34 specimens with no growth, as indicated in figure (1). The findings showed that 17 (65.3%) specimens were Gram negative bacteria and that 9 (34.7%) specimens were Gram positive isolates. 2 (7.7%) of the total 34.7% of specimens had the Gram-positive isolate S.xylosus.

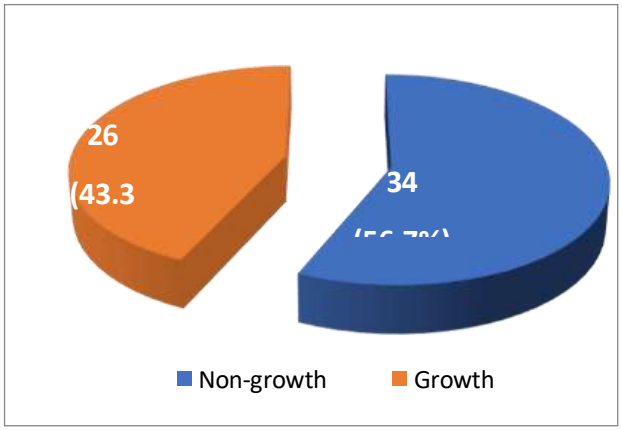


*Figure 1: shows the proportion of bacterial growth*

### Cultural and Morphological Characteristics

As depicted in figure (2), S.xylosus colonies on blood agar are large, raised to slightly convex, circular, smooth to gritty, opaque, dull to gleaming, with some.

### Colonies pigmented white yellowish or yellow-orange

*Figure 2: S.Xylosus on blood agar*

As shown in Table (1), all isolates staphylococcus showed negative results to oxidase, motility and urease tests, and positive to catalase. The coagulase test revealed that all *S.aureus* was positive and all staphylococcus and *S.xylosus* was negative to this test.

**Table 1: Biochemical test of S.xyluses isolates**

| Tests | Result |
|---|---|
| Gram stain | + |
| Catalase | + |
| Coagulae | - |
| Hemolysin | - |
| Growth on mannitol agar | + |

**Immunological Study**

The anti-dsDNA antibody level was found to be higher in patients with SLE S.xylosus infection (15.23±0.05 IU/ ml) compared to the control group (7.25 ± 0.09 IU/ml) using the ELISA, which was used to assess several forms of autoantibodies. Additionally, it has been discovered that individuals with SLE had the greatest levels of ANA antibodies (1.14±0.09IU/ml), compared to healthy people (0.4±0.08 IU/ml), as shown in table (4). However, ds-DNA and ANA autoantibodies in SLE patients with S.xylosus infection are within the normal range of human health. Using the radial immunodiffusion assay to measure the level of serum compulsion C3, C4, the results revealed a significant decrease (P>0.05) in the serum of SLE patients with S.xylosus infection (85.41 ± 2.5,18.79 ± 1.9mg/dl, and 36.21.99mg/dl, respectively) when compared with healthy individuals.

**Table 2: Concentration of autoantibodies and complements in SLE patients with s.xylosus and healthy controls**

| Parameters | SLE with S.xylosus | Healthy controls | P-value |
|---|---|---|---|
| **ANA** | 1.14±0.09 | 0.4±0.08 | 0.0001* |
| **Anti ds-DNA** | 15.23±0.05 | 7.25±0.09 | 0.015* |
| **C3** | 85.41±2.5 | 105.5±2.11 | 0.001* |
| **C4** | 18.79±1.9 | 36.2±1.99 | 0.0001* |

**Haematological study**

The results of the statistical analysis of the study shown in Table (3) The data of this study have indicated a significant decrease in levels of HB, WBC and platelet (9.35±0.1, 3.34±0.7 and 142.6±2.1) respectively and increase ESR levels among SLE patients with S.xylosus (60.4±2.86) compared with control.

**Table 3: Haematological parameters**

| Haematological parameters | SLE patients with S.xylosus | Healthy controls | P-value |
|---|---|---|---|
| HB (g/dl) | 9.35±**0.1** | 12.14±**0.09** | **0.0001*** |
| WBC (x103/uL) | 3.34±**0.7** | 9.25±**0.8** | **0.011*** |
| Platelat (x103/uL) | 142.6±**2.1** | 321.2±**2.24** | **0.002*** |
| ESR | 60.4±**2.86** | 12.8±**2.1** | **0.006*** |

**Measuring the level of cytokines in the serum of SLE patients with with S.xylosus infection (Cluster of differentiation 69 (CD69)**

The results of the statistical analysis of the study are displayed in Table (4) as a significant increase of P<0.05 in the level of CD69 in the serum of S.xylosus- infected patients compared to healthy serum levels. The serum CD69 levels were (14.089±0.25ng/ml) compared to (10.965±1.06 ng/ml) in healthy individuals.

**Cytokine IL-21, IL-35**

The results indicate a significant increase ($P \leq 0.05$) in the serum level of IL-21 and IL-35 in the all of SLE patients infected with S.xylosus (12.896±0.13 pg/ml ,15.32±0.11pg/ml) respectively, compared with its levels in healthy serum (8.0703±0.08, 12.142±0.09pg/ml) respectively. This show in table (4).

**Table 4: Concentration of some cytokines in SLE patients with s.xylosus and healthy controls**

| Cytokines | SLE patients with S.xylosus | Healthy controls | P-value |
|---|---|---|---|
| **CD69** | 14.089±0.25 | 10.965±1.06 | 0.0001* |
| **IL-21** | 12.896±0.13 | 8.0703± 0.08 | 0.0001* |
| **IL-35** | 15.32±0.11 | 12.142±0.09 | 0.0001* |

Several systems are affected by the autoimmune, inflammatory condition known as systemic lupus erythematosus (SLE), which also exhibits a wide range of clinical symptoms. Numerous organ systems are impacted, and the severity of the disorder varies; some people just suffer rashes and arthralgias, while others have significant multi-organ involvement with nephritis or vasculitis (Speyer *et al*., 2020). People with systemic lupus erythematosus (SLE) are more vulnerable to infections, which can lead to serious complications and even death (Cervera *et al*., 2003). Numerous research has investigated the prevalence of infection in SLE and its associated clinical and laboratory features. Leukopenia, steroids, immunosuppressive drugs, and the length, intensity, and duration of the disease have all been linked to numerous concoctions that increase the chance of infection (Gladman *et al*., 2011).

In the investigation, gram-positive isolates of S.xylosus were found in 2 (7.7%) of the total 34.7% of samples. A previous study (Smith & Cyr, 1988). Found that Staphylococcus spp. are often isolated from the oral cavity in both healthy persons and patients with systemic illnesses. In both groups, the prevalence of staphylococci was similar (94%) to that for healthy individuals described in the literature (de Araújo *et al*., 2012). The two most prevalent species isolated were S. epidermidis and S. aureus were most frequently found in the oral cavity. Though caution should be used because some drugs might increase the risk of staphylococcal infection, staphylococcus spp. colonization did not vary between SLE and controls. Given that studies suggest that Staphylococci have the ability to disseminate illnesses to remote regions, these individuals may be at danger because of this threat (Kralovic *et al*., 1995).

S. xylosus infections in humans have sporadically been reported. The literature currently only has a few number of instances, such as secondary root canal infection (Siqueira & Lima, 2002), that indicate S. xylosus' pathogenetic involvement. There is ear obstruction (Akhaddar et al., 2010). orthopedic implants that have infections (Arciola et al., 2006). the esophagus eroding (Chervinets, 2002). Numerous coagulase-negative staphylococcal species were recovered from individuals with nosocomial bacteremia, S. xylosus being one of them (Koksal et al., 2009). also, from a variety of clinical samples, such as blood, tracheal aspiration, and urine (Boynukara et al., 2007). Anti-dsDNA

and anti-ANA antibodies were both higher than they had been in the control group., but the autoantibodies of SLE patients with S. xylosus infection were within the normal range of health in humans and Erythema nodosum associated with Staphylococcus xylosus septicemia had normal ds-DNA and ANA levels.

Infections with microorganisms may also advance the onset of SLE. Any bacterially generated inflammation results in cellular damage and raises the amount of cellular debris, activating B lymphocytes or encouraging the generation of autoantibodies (Green & Marshak, 2011). By way of certain substances like lipopolysaccharides or immune complexes containing nucleic acid, bacterial infections trigger the immune system to respond. PAMPs interact with internal TLR and non-TLR receptors on antigen-presenting cells, monocytes, and B and T lymphocytes. TLR activation causes plasmacytoid dendritic cells to produce IFN, which in turn causes the generation of pro-inflammatory cytokines and the breakdown of innate immune mechanisms (Francis & Perl, 2010). An important factor in the aetiology of systemic lupus erythematosus (SLE) may be dysregulated lymphocyte subpopulation ratios and aberrant immune cytokine production, both of which are associated with SLE paroxysms (Hashemi & Malarkannan, 2020a), according to some research.CD69 is expressed on the cell surface as a result of T lymphocyte activation. Furthermore, effector activities are activated and induced in cells that carry this receptor when particular antibodies cross-link CD69. However (Hashemi & Malarkannan, 2020b), it is uncertain what CD69's physiological ligand is. CD8 cells from patients were shown to have higher levels of the CD69 antigen compared to controls.

This shows that when examined in an experimental setting, cytotoxic T lymphocytes linked to SLE are already triggered (active in vivo). This aberrant CD69 expression may contribute to the continuing inflammatory response by allowing autoreactive cells to proliferate unchecked. Consistent with previous findings in SLE patients (Wang *et al*., 2019), we discovered that CD69 expression was elevated in newly separated CD4 and CD8 peripheral blood cells, but only in the CD8 fraction. CD69, a type II transmembrane glycoprotein related to C-type animal lectins, is one of the earliest acquired specific antigens during lymphoid activation. It is expressed and demonstrated regulated expression on numerous hematopoietic lineage cells, including neutrophils, monocytes, T cells, B cells, natural killer (NK) cells, and platelets (Koyama *et al*., 2022). In SLE patients as opposed to healthy controls, plasma concentrations of IL-21 are much greater and are correlated with Tfh cell numbers. illness severity and illness indicators, such as serum C3 complement fraction and SLEDAI scores, are associated with blood levels of IL-21.

Over time, SLE patients were shown to have more CD4+ T cells that expressed IL-21 than healthy individuals 34. When it comes to T cell and B cell responses in SLE, IL-21 may be a key modulator (Terrir *et al*., 2012). The hallmarks of systemic lupus erythematosus (SLE) include autoantibody generation, loss of peripheral and central immunological homeostasis (tolerance-promoting B and T cells), and organ damage. This study set out to identify the immune-regulatory profile of IL-35 and its soluble receptors in peripheral blood mononuclear cells (PBMC) from SLE patients with different disease activity indices (SLEDAI). Patients with SLE had substantially higher IL- 35 titers than healthy controls. The percentage of peripherally circulating Treg cells and disease activity in SLE patients were shown to be in direct opposition to one another, according to the scientists. In individuals with severe SLE, a greater Teff to Treg cell ratio (Teff; Treg) was also seen (Cai et al., 2015). Therefore, it is necessary to conduct tests to identify bacterial infections in patients, and rely on CD69, IL-21and IL-35 to diagnose the SLE disease.

## CONCLUSION

S.xylosus give nommal autoantibody levels used in diagnosing SLE. The data of this study have indicated significant decrease in levels of C3, C4, Hb,WBC and platelet but elevated ESR compare

with control. A high concentration of inflammatory cytokines cold plays a crucial role in pathogenesis of SLE that leads to development of disease.


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## CONFLICT OF INTEREST

There was no conflict of interest in this study.


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