

Research Article

Impact of Interleukin-37 and Vitamin D on children with *Streptococcus pyogenes* sore throat in Salah al-Din Governorate, Iraq

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Abstract

Sore throat is a common presentation in pediatric office settings and emergency departments. Bacterial infection due to *Streptococcus pyogenes* represented about 25% of sore throat in children. The study aimed to find a relationship between interleukin-37 and vitamin D levels in children with sore throat infection by *S. pyogenes* bacteria from Salah Al-din General Hospital and Samarra General Hospital in Salah al-Din Governorate, Iraq. The number of sore throat patients in the present study was 317, aged between 5 and 17 years old. The control group included 80 healthy individuals. Throat swabs from children were collected and aseptically inoculated on Petri plate media. Blood samples were collected from patients and control children for determination of Vitamin D and IL-37 by ELISA. The study showed that 30.28% (96 of 317) of the children studied were infected with *S. pyogenes* bacteria. The results revealed a significant elevation in the mean IL-37 level among children with *S. pyogenes* infection (96.9 ± 12.2 pg/ml) compared to the control group (62.5 ± 11.3 pg/ml) (p -value = 0.0001). Furthermore, children with *S. pyogenes* infection had a significantly lower mean vitamin D level (22.3 ± 6.1 pg/ml) compared to the control group (52.8 ± 3.08 pg/ml) (p -value = 0.0001). In conclusion, this study provides evidence of significant alterations in immune markers, including IL-37, and vitamin D levels, in children with *S. pyogenes* infection compared to the control group. These findings suggest the involvement of these immune markers in the pathogenesis of *S. pyogenes* infection.

Keywords: Interleukin-37, Vitamin D, *Streptococcus pyogenes*, Sore throat

INTRODUCTION

Sore throat is a common presentation in pediatric office settings and emergency departments. Bacterial infection due to *Streptococcus pyogenes* represented about 25% of sore throat in children (Abd Al-Kareem *et al.*, 2014). This is a major human-specific bacterial pathogen that causes a wide array of manifestations ranging from mild localized infections to life-threatening invasive infections (Hadi *et al.*, 2017). Group A of streptococci (GAS) pharyngitis is most common among 5–14-year-old children and causes around 37% of all pharyngitis episodes in this age group. The global burden of severe *S. pyogenes* infections is 18.1 million cases with 1.78 million new cases per year (Miller *et al.*, 2022). The analysis of epidemiological data indicates a correlation between vitamin D deficiency and an increased susceptibility to streptococcal infection and related disease outcomes. Furthermore, vitamin D has been found to

enhance the body's antibacterial defenses by activating innate immune mechanisms, such as phagocytosis. Additionally, it has been observed that vitamin D promotes the production of reactive oxygen species, known as oxidative burst, as well as antimicrobial peptides, including cathelicidin and lactoferrin. These components are crucial in effectively eliminating bacteria (Syed *et al.*, 2020; Auala *et al.*, 2022; Murdoch *et al.*, 2019). Interleukin-37 (IL-37) is a recently discovered cytokine that belongs to the interleukin-1 family. Numerous studies have provided evidence showcasing the immunosuppressive properties of IL-37, which effectively mitigates innate and acquired immune responses by inhibiting various inflammatory mediators (Ismailova *et al.*, 2022 and Allam *et al.*, 2020). The aim of the present study was to evaluate the impact of serum levels of Interleukin-37 related to vitamin D in sore throat patients infected with *S. pyogenes*.

MATERIALS AND METHODS

A cross-sectional study was carried out in Salah Al-din Governorate from the 15th of December 2022 to 15th of March 2023. The number of sore throat patients in the present study was 317, aged between 5 and 17 years old, from Samarra General Hospital, Salah Al-din General Hospital, private clinics and health care centers. The control group matched to patients studied included 80 healthy individuals with no acute and chronic diseases, and their ages were between 5 and 17 years old. These individuals were in the same hospitals and health care centres.

Collection of samples

The samples were taken from the throats of children by rubbing the sterile swab tip on the surface of one or both tonsils, the tonsillar pillars, or the posterior pharyngeal wall without touching the teeth, gums or tongue. The swabs were aseptically inoculated on Petri plates containing blood agar and MacConkey media. After incubation, the colonies were morphologically and microscopically examined. The colonies were subculture on solid media several times and preserved at 4 °C for further investigations. Microorganisms were identified on the basis of their morphological characteristics on selective and differential media. API Strep kits were used for biochemical testing to confirm the identity of microorganisms.

Five ml of blood was collected by vein puncture using a five ml syringe from each patient and control persons. Blood samples were placed into in plane tubes, left for 30 minutes at 37 °C for clotting and centrifuged at 3000 rpm for 15 minutes, sera from were then aspirated and transferred into Eppendorf tubes for determination of Vitamin D and IL-37 by Enzyme linked immunosorbent assay (ELISA) (Banerjee *et al.* 2020)

Diagnosis

The bacteria growing on the media were diagnosed based on color, shape, size, edge and height of the growing colonies, and the colonies were regrown more than once to obtain single pure cultures, and the cultivation characteristics of the growing colonies were established on the following culture media (Blood agar, MacConkey agar, and Mannitol salt agar).

Microscopically examination

Gram stain reaction was done by placed loopful of distilled water in the ringed area of the slide, a small amount of the bacteria was mixed in the water and spread out. The smear was air dried and then heated by passing the slide rapidly through the burner flames three times after that the smear was stained with gram stain and examined under oil immersion for *S. pyogenes*, which appeared as Gram-positive cocci, arranged in chains (Torres Inuma, 2020)

API (Analytical Profile Index) 20 Strep Test

The API strep consisted of 20 microtubes containing dehydrated substrates. These microtubes were inoculated with a bacterial suspension and prepared in an API strep medium that reconstitutes the tests. During incubation, metabolism produces color changes that were either spontaneous or revealed by adding reagents. The reactions were read according to the Reading table and the identification was obtained by referred to the Analytical Profile Index.

The microtubes were filled with the inoculated API strep medium using a pipette. Only the tube portion of the microtubes was filled. To avoid the formation of bubbles at the base of the tubes, the strip was tilted slightly forward, and the tip of the pipette placed against the side of the cupule. Anaerobiosis in the tests was certified by filling the cupules with mineral oil to form a convex meniscus. The incubation box was closed and incubated at 36 °C ± 2 for 18-24 hour (Spellerberg and Brandt, 2022). This test was used to confirm the initial diagnosis of *Streptococcus ssp.* and differentiated between groups A,B,C,G.

Statistical analysis

Computerized statistical analysis was performed using IBM SPSS ver 23.1 statistic program. Comparison was carried out using T-Test. The *P. value* > 0.05 was considered statistically significant, while for those which its *P. value* > was greater than 0.05 considered non-significant statistically.

Ethical approval

Ethical approval was obtained from the Scientific Research Ethics Committee at the College of Medicine, Tikrit University, No. 3/7/515 on 28/11/2022.

RESULTS AND DISCUSSION

Sore throat is a common complaint in Pediatric offices and Emergency Departments Samarra city in Iraq, with viral infections being the most common cause (Uğur and Yüksel, 2023).

According to the current study, (figure 1) 30.28% (96 of 317) of the children under investigation had *Streptococcus pyogenes* infection, 14.20% Group G *Streptococci milleri*, 6.94% had *H. influenzae*, 8.52% had *S. aureus*, 10.73% had *Escherichia coli*, and 3.15% had *Klebsiella pneumoniae*, while 17.03% had negative growth on throat swabs. The current results were consistent with a number of Iraqi research (Abd Al-Kareem *et al.*, 2014; Hadi *et al.*, 2017) that demonstrated the role of group A beta-hemolytic streptococci (GABHS) in tonsillitis, as demonstrated by positive cultures of these microorganisms. The current findings were also in agreement with the results reported by Darod *et al.* (2022), who discovered that 23 (19.2%) of the 120 (32.1%) children who

Table 1. Levels of Interleukin-37 and Vitamin D in children with *S. pyogenes* infection and control group

Studied groups	No. of patients	IL-37 (pg/ml) Mean \pm SD	P-Value	Vitamin D (ng/ml) Mean \pm SD	P-Value
Streptococcus pyogenes patients	96	96.9 \pm 12.2		22.3 \pm 6.1	
Control group (Without Streptococcus pyogenes)	80	62.5 \pm 11.3	0.0001	52.8 \pm 3.08	0.0001

tested positive for bacterial throat cultures were also mixed isolates. The most common types of bacterial isolates were *Staphylococcus aureus* (42, 29%), *S. pneumoniae* (10), and beta-hemolytic streptococci (78, 55%).

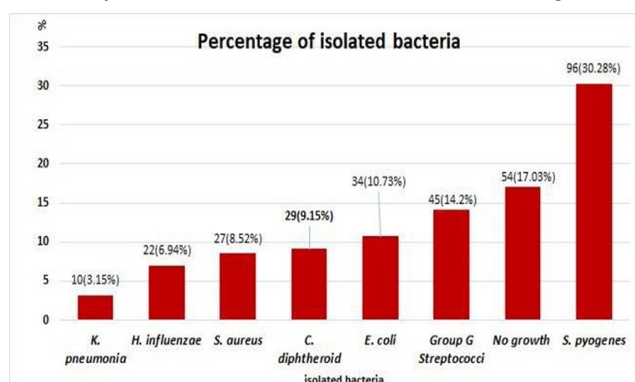
There are several reasons why *S. pyogenes* may have the highest percentage occurrence among bacteria isolated from throat swabs in the present study. *S. pyogenes* can spread easily through respiratory droplets when an infected person coughs or sneezes. This makes streptococcal infections highly contagious, leading to a higher occurrence rate (Darod *et al.*, 2022).

As shown in Table 1 with a P-value of 0.0001, the study revealed that children with *S. pyogenes* infection had a substantially lower mean vitamin D level (22.3 \pm 6.1 pg/ml) compared to the control group (52.8 \pm 3.08 pg/ml). When it comes to immune system modulation, vitamin D is essential. It has been demonstrated to improve both adaptive and innate immune responses and the synthesis of antimicrobial peptides. The immune system's reaction to the bacterial invasion in *S. pyogenes* infection may result in increased vitamin D utilisation and depletion (Prameshti *et al.*, 2021). A lack of vitamin D increases the likelihood of infection, and supplementing with the vitamin changes how the host and pathogen interact. Micronutrient deficits, such as inadequate vitamin D, have been linked to a higher risk of infection, particularly *Streptococcus pyogenes*-related infections. Tonsillopharyngitis and community-acquired streptococcal pyogenes have been linked to vitamin D insufficiency (Guevara *et al.*, 2021). As far as we currently know, this study looked at the possible link between vitamin D and *S. pyogenes* in kids. The current research suggests a connection between vitamin D deficiency and sore throat recurrence. According to Aydin *et al.*, children with recurring sore throats were likelier than healthy children to have insufficient vitamin D (Aydin *et al.*, 2021).

The investigation found that children infected with *S. pyogenes* had a mean IL-37 level that was significantly higher (96.9 \pm 12.2 pg/ml) than the control group (62.5 \pm 11.3 pg/ml), with a P-value of 0.0001 (Table 1). The current findings showed that tonsillitis patients' blood IL-37 levels increased considerably compared to the healthy control group. The dynamic human cellular immune response to *S. pyogenes* infection is well characterized in this work. Acute pharyngitis is characterized by elevated monocyte and dendritic cell subsets,

elevated IL-37, activation of unconventional T cells, and blood-borne migration of B cells and CD4+ T-cell subsets. The immunological signature of *S. pyogenes* will now be followed throughout the research landscape, starting with samples from in vitro experiments and real human infections (Anderson *et al.*, 2022). The current work is consistent with that conducted by Uhlmann *et al.* (2016), who found that although the antimicrobial peptide LL-37 is widely expressed; its bactericidal impact against *S. pyogenes* infections is minimal. The elevated pro-inflammatory activity raises the possibility that LL-37 plays a role in the pathophysiology of *S. pyogenes*. According to several studies, IL-37 levels were quickly raised in response to inflammation in order to stop excessive tissue damage brought on by the host immune system's defenses against microbial infections (Banchereau *et al.*, 2012).

Of 96 patients with *S. pyogenes*, 54(56.25%) cases were in the age group between 5-8 years, 25% were between 9-12 years old, and 18.75% were between 13-17 years old. This distribution suggests that children in the 5-8-year age group had the highest occurrence of *S. pyogenes* infection, followed by the 9-12-year age group and then the 13-17-year age group as shown in Fig. 2. These results agree with finding of Abd Al-Kareem *et al.* (2014) found that most children with *S. pyogenes* infection were below 10 years old. The finding of an age-related susceptibility to *S. pyogenes* infection suggests that certain age groups may be more prone to contracting infections caused by these specific bacteria. The immune system undergoes changes and maturation as individuals age. Young children, particularly infants and toddlers, may have an immature immune system that is less efficient at combating infec-

**Fig. 1.** Isolated bacteria from throat swabs of studied children

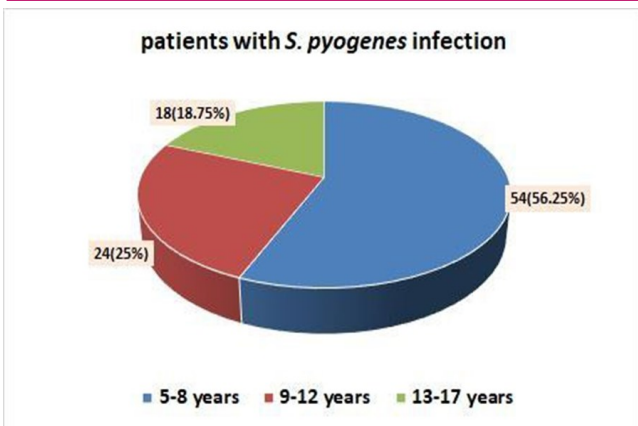


Fig. 2. Distribution of patients with *S. pyogenes* infection according to age

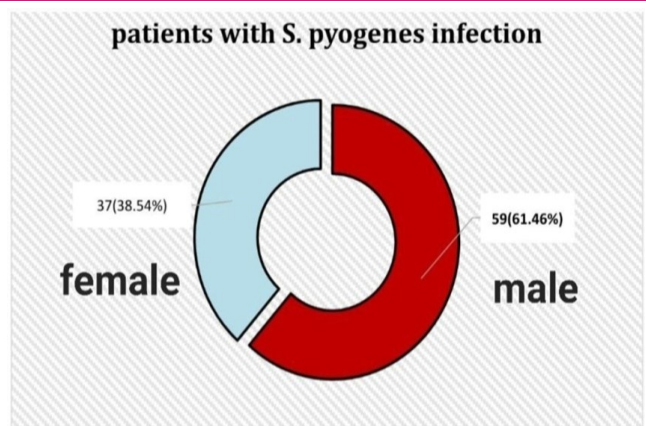


Fig. 3. Frequency of children with *Streptococcus pyogenes* infection according to sex

tions. This could make them more susceptible to *S. pyogenes* infection than older children or adults with a more developed immune response (Lee *et al.*, 2017). The study findings indicate that 59 (61.46%) of the children diagnosed with *S. pyogenes* infection were male, while 37 (38.54%) were female, as presented in Fig. 3. These results correspond with Abd Al-Kareem *et al.* (2014) who found that most children with *S. pyogenes* infection were males. Kalaf (2022) also found that male children were more frequently infected with *S. pyogenes* than females. This gender distribution raises several potential factors and considerations that could contribute to such a difference. There were some possible explanations: Differences in susceptibility to *S. pyogenes* infection between males and females may be influenced by biological factors such as variations in hormonal profiles or immune responses between sexes could play a role.

With rates of 87.5%, 83.33%, 81.25%, 71.87%, 68.75%, and 65.63%, respectively, Fig. 4 illustrates the large proportion of *S. pyogenes* isolated from children

with throat infections that were sensitive to cefepime, vancomycin, ceftriaxone, carbenicillin, clindamycin, and co-trimoxazole. However, a significant proportion of resistance to ampicillin (79.17%) and amikacin (60.42%) was observed. Edrees and Anbar (Edrees and Anbar, 2021) discovered that *S. pyogenes* had the highest recorded sensitivity to vancomycin, ceftriaxone, and carbenicillin, and that the proportion was less susceptible to ampicillin. These findings are consistent with the current studies.

Mahdi (2009) discovered that carbenicillin, ceftriaxone, vancomycin, and cefepime had the highest sensitivity. The percentages of *S. pyogenes* that were isolated (80.6%) and how sensitive they were to cefotaxime and cephalexin (72.5%) and how resistant they were to amikacin (100%), respectively, were comparable to the findings reported by Kebede *et al.* (2021). These results may be interpreted in a way that considers the contents, which suggested that cefepime was used more recently in those years, that usage had decreased after a while, and that subsequent re-use had caused bacte-

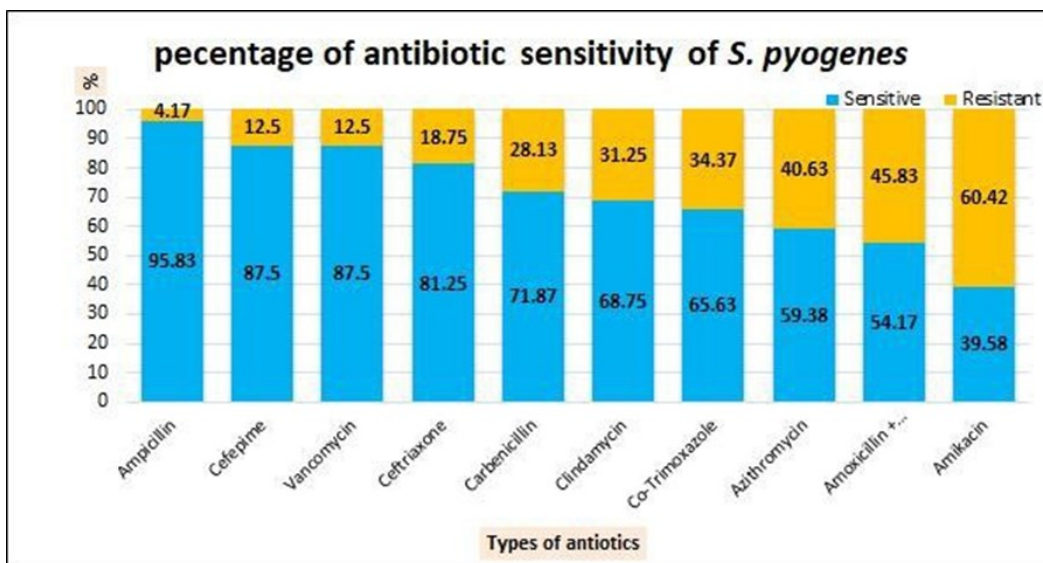


Fig. 4. Antibiotics sensitivity *Streptococcus pyogenes* infection isolated from children with throat infection

ria to react quickly. Because bacteria that are resistant to the formation of enzymes can modify antibiotics and lose their effectiveness, or because the loss of outer membrane proteins reduces the permeability of the antibiotic within cells, there could be two possible explanations for the high resistance to amikacin based on the presence of enzyme formation (Meletis *et al.*, 2023). The current research suggests that the underlying causes of recurrent pharyngitis, sore throats, and *S. pyogenes* bacterial infections include immunodeficiency and vitamin D insufficiency. The host immune system's responses against microbial infections caused substantial tissue damage, which is why IL-37 levels were swiftly increased in response to inflammation. It is possible that *S. pyogenes* infection may be more prevalent in specific age groups, 5-8, due to the likelihood of an age-related sensitivity to these particular bacteria. People's immune systems change and adapt as they age.

Conclusion

In conclusion, this study showed a relatively high prevalence of this pathogen among young children aged between 5-8 years and males more susceptible to *S. pyogenes* infection. High percentage of *S. pyogenes* isolated from children with throat infection were susceptible to cefepime, vancomycin, ceftriaxone, carbenicillin, clindamycin and co-trimoxazole and a high rate of resistance was recorded toward amikacin and ampicillin. Low vitamin D levels have been associated with increased susceptibility to infections and impaired immune responses. Also, this study showed a significant positive correlation of IL-37 with each of anti-DNase and ASO levels among children with *S. pyogenes* infection and a positive correlation of Anti DNase levels ASO and a negative correlation with Vitamin D among children with *S. pyogenes*. Further, the study is warranted to explore the underlying mechanisms and potential therapeutic implications of these immune marker alterations in *S. pyogenes*-infected children.

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Conflict of interest

The authors declare that they have no conflict of interest.

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