



Rafidain Oral and Dental Research Journal

Journal homepage <https://rodri.edu.iq>



Association Between ABO Blood Groups and Salivary TNF- α Levels Among Patients with Viridans Streptococci Oral Infections in Baghdad

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ARTICLE INFO

Article history:

Received 16-07-2026
Revised 16-07-2026,
Accepted 26-06-2026,
Available online 27-07-2026

Keywords:

Oral infection,
Viridans Streptococci,
TNF- α ,
ABO blood group.

ABSTRACT

Background: Oral infections are bacterial, fungal or viral diseases affecting the teeth and oral mouth tissues. include cavities, (gingivitis/periodontitis). These bacterial infections lead to increased cellular immunity and cytokines included the immunological evaluation of the TNF- α cytokine in the saliva of oral cavity patients infected with bacteria,

Aim: this study aimed to investigate the association between *Viridans Streptococci* oral infections, ABO blood groups and evaluation the TNF- α cytokine in saliva.

Materials and Methods: During the period from (November 2024) until (June 2025), blood and saliva samples were collected from 150 participants who were Al-Karama Specialized Dental Center in Baghdad, their ages range from 18 to 55 years, both male and female and have no prior medical history. divided (100) clinical samples were for *Viridans Streptococci* infection, 50 blood and 50 saliva samples were from control individuals.

Diagnosis of bacteria were confirmed by complete biochemical examinations using VITEK2 Compact System. Assessments included the saliva level of cytokine (TNF- α) for patients infected with *Viridans Streptococci* who visit Al Karama Specialized Dental Center.

Results: The study recorded a significant increase in the saliva level of TNF- α for patients infected with *Viridans Streptococci* (95.05 ± 25.90) pg/ml compared to control subjects (24.11 ± 1.35) pg/ml.

Differences were observed in the percentages of phenotypic patterns of ABO blood groups among patients compared to control individuals, as individuals with blood type O showed more predisposition to infection compared to other blood groups, while blood group AB had the lowest rate of infection compared to other blood groups.

Conclusion: The Result an association was observed a correlation between different blood types and infections with *Viridis streptococci* in the oral tissues of infected individuals, as well as an elevated TNF- α index in the saliva of patients infected with this bacterium.

1. Introduction

The oral cavity is a specialized ecological niche of the human body and forms a continuum with the digestive and respiratory system (1).

A common oral disease of polymicrobial etiology is dental caries, whose incidence and progression rate has declined in recent years due to successfully introduced population-based prevention measures (2,3), oral infection

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due to the precipitation of bacteria and debris between line of gingiva and tooth, account for up to 90% of cases (4). The largest bacterial communities found in the mouth that are considered normal flora are streptococcal bacteria. (5)

Viridans Streptococci, are a large group of commensals, Gram-positive, alpha-hemolytic bacteria commonly found in the human mouth, respiratory tract, and gastrointestinal tract, that are commonly associated with infective endocarditis (IE) (6), While generally harmless, they can cause serious conditions like dental caries and subacute infective endocarditis (7), *Viridans streptococci* are identified in cases of neonatal infections. (8)

The *Viridans* group of bacteria includes various types, some normal flora and less them pathogenic, such as: *S. anginosus* , *S. mitis* ,*S. sanguinis* , *S. bovis* ,*S. salivarius* and *S. mutans* (9).

Identifying viridans group streptococci (VGS) can be challenging, phenotypic identification is not always accurate, and the term "viridans" is somewhat imprecise. Not all species cause hemolysis in blood culture media, and incubation temperatures vary. No enzymatic or toxin-producing effects have been documented as byproducts of alpha-hemolysis. (10,11)

Viridans streptococci are a major cause of endocarditis and typically infect patients with underlying valvular disease. They can also cause bacteremia (especially in neutropenic patients), meningitis, and pneumonia. (12)

Viridans streptococci, particularly the *S. mitis* and *S. salivarius* groups, are resident microorganisms in the oral and pharyngeal cavities of all age groups. They inhibit colonization by many pathogens, including *Streptococcus pyogenes*. This is achieved through two distinct mechanisms: bacteriocin production and hydrogen peroxide production (which is also responsible for alpha-hemolysis). (13,14)

Viridans streptococci were once completely sensitive to penicillin, but penicillin resistance has increased, particularly in hospital-acquired bloodstream isolates from immunocompromised patients. Synergistic bactericidal activity has been observed with the use of penicillin and aminoglycosides. Therefore, a combination of penicillin and gentamicin is commonly used to treat endocarditis. (15)

Recently, there has been considerable interest in the relationship between ABO blood types and systemic diseases (16, 17), Several previous studies have revealed significant variations in the relationship between ABO blood type and periodontal disease, which may be important in developing important and early treatments that may help determine why susceptible individuals do not respond to oral diseases caused by different types of bacteria. (18).

Specific antigens on the surface of blood cells determine the four main blood types: O, A, B, and AB. These antigens are present in the bodily secretions of most individuals, who are then termed "sorters." In contrast, non-sorters lack these antigens in their bodily secretions. (19)

This distinction is important because non-sorters generally exhibit lower levels of immunoglobulin A (IgA) antibodies in their saliva, which may impair their ability to fight off bacterial populations in the oral cavity. Furthermore, the presence of blood type antigens in saliva may inhibit bacterial adhesion to tooth surfaces, as many oral bacteria possess lectins that specifically bind to blood type antigens. (20)

Research investigating the relationship between blood type and tooth decay has yielded mixed results. Some studies have suggested that individuals who secrete blood antigens in their saliva have a lower prevalence of tooth decay compared to those who do not, possibly due to the inhibitory effects of salivary antigens on bacterial adhesion (21, 22)

Despite this research, no definitive studies have established a statistically significant relationship between salivation status or blood type and prevalence. (23), These discrepancies may be attributed to the geographical and demographic differences among the study populations. (24).

Tumor necrosis factor- α (TNF- α), also called TNF ligand superfamily member 2 (TNFSF2), is a pro-inflammatory cytokine that is mainly produced by activated monocytes and macrophages in response to infection, injury, and tumor burden, (25).

TNF- α production has also been found in a range of other inflammatory cell types, including T cells, NK cells, and neutrophils, as well as non-immune cells including keratinocytes and astrocytes. (26). It is also recognized that TNF- α is not only involved in tissue inflammation and injury, but also appears to be a prominent ligand for the activation of programmed cell death through apoptosis. (27)

Despite previous studies investigating the association between ABO blood groups and oral diseases, as well as the role of TNF- α in inflammatory responses, limited information is available regarding the association between ABO blood groups and salivary TNF- α levels in patients with oral infections caused by *Viridans streptococci*.(28)

We hypothesized that ABO blood groups may influence susceptibility to *Viridans streptococci* infection and the associated inflammatory response, reflected by changes in salivary TNF- α levels.

Therefore, this study aimed to investigate the association between ABO blood groups, *Viridans streptococci*-associated oral infections, and salivary TNF- α levels among patients in Baghdad.

2. Materials and methods

2.1. Ethical considerations

This research protocol was reviewed and approved by the Research Ethics Committee in Al Rafidain University college, Department of

Dentistry, the protocol date of approval was 15/10/2024. All participants were informed about the aims of the study, and had read and signed an informed consent form

2.2. Populations studied

150 blood and saliva samples, divided (100) blood and saliva,(50)control from healthy individuals, samples were collected from patients admitted to patients attending Al-Karama Specialized Dental Center in Baghdad their ages range from 18 to 55 years, both male and female and have no prior medical history infected with *Viridans streptococci*, For the purpose of diagnosing blood group and bacterial *Viridans streptococci* infection. where was collected (1 ml) of venous blood was taken from each of them and (5 ml) of saliva, after obtaining their consent, for immunological examinations.

Viridans streptococci, was isolated from infected patients and diagnosed by traditional methods (using different culture media (MacConkey agar, blood agar) and by biochemical assays), then the bacteria diagnosis was confirmed using VITEK 2 ID-GP cards.

2.3. Identification of *Viridans streptococci*, via VITEK2 Compact System

The bacterial isolate was identified using the VITEK2 technology. On MacConkey agar dishes, the bacterial isolates were sub cultured. Bacterial suspensions in 0.45 percent sterile NaCl solution were employed, which were similar to MacFarland 0.5x 10⁸ standards. A densitometer was used to regulate the turbidity of the bacterial suspension. The VITEK 2 compact system was manually loaded with VITEK 2 ID-GP cards, AST-No. 12 cards, and bacterial suspension. A bacterial suspension was physically put into each test card, sealed, and incubated for 6 hours. During this time, the cards were recited every 15 minutes using kinetic fluorescence measurement. The VITEK 2 compact system software examined the data first, then mechanically reported the results. (29)

The Viridans group of bacteria includes various types, some normal flora and less them pathogenic, such as: *S. mitis* ,*S. sanguinis* , *S. salivarius* and *S. mutans*.

2.4. Assessments of saliva levels TNF-alpha

Saliva levels of TNF-alpha were detected using commercial kits, which were products of KOMA ELISA (Labiskoma, Korea). The two groups were based on similar principles; Therefore, methods for assessing saliva markers were mentioned simultaneously.

The kits methods were based on the principles of sandwich enzyme-linked immunosorbent assay (ELISA). In which the wells of the microplate were pre-coated with a specific anti-marker antibody (Capture antibody: anti-human TNF-alpha). Upon adding standards or saliva samples to the appropriate wells, a reaction occurs with the specific antibody. This step is followed by adding horseradish peroxidase (HRP)-conjugated with the specific antibody. After a period of incubation, it is followed by a washing step, the TMB substrate solution (3,3',5,5'-tetramethylbenzidine) is added to each well. At this point, the blue color is developed in the wells, and after adding the stop solution, the color turns yellow. The density of the color is proportional to the level of TNF-alpha. At a wavelength of 450 nm, optical density (OD) is measured spectrophotometrically.

2.5. ABO blood group examination

The ABO and Rh blood types were determined using the agglutination method. When red blood cells carrying one or both antigens are exposed to the right antibodies, they interact and cause clumping or agglutination to occur. (30) ABO blood group antigens are O linked glycoproteins in which the terminal sugar residues exposed on the red blood cell surface determine whether the antigen is A or B. People with blood group A have antigens on Erythrocytes and anti-B antibodies in their serum. (31) People with blood group B also have B antigens on Erythrocytes and anti-A antibodies in their serum. Antigens from A and B are present on Erythrocytes of people with blood group AB, but no anti-A or anti-B antibodies are present in their blood. Although people with blood group O lack A and B antigens, their serum contains anti-A and anti-B antibodies. (32-33).

2.6. Statistical analysis

The mean $\pm$ SD of mean was calculated by using the IBM SPSS version 26.0 (34) The probability was also examined by using student T-test and statistical significance was considered at $P \leq (0.01)$. The WinPepi application version 11.65 was used to calculate the probability for non-parametric data using Pearson's chi-square test. (35)

3. Results

3.1. The results of bacterial diagnostic tests on culture media and some biochemical tests were as noted in the table (3-1) below.

Table (3-1): Results of Biochemical Identification tests of *Viridans streptococci* isolates.

No.	Biochemical tests	Result
1	Catalase production	(-)
2	Citrate Utilization	(-)
3	Hemolysin production	(+)
4	H ₂ S production	(-)
5	Lactose fermentation with gas production	(+)
6	Motility	(-)
7	Oxidase production	(-)
8	Sucrose and glucose fermentation	(+)

(+) = Positive (-) = Negative

3.2 Identification of *Viridans streptococci* by VITEK 2 system

Most of the samples that were isolated as *Viridans streptococci* by using VITEK 2 system with its identification card for Gram Positive strains (ID-GP). This system has been employed in a number of previous research and has produced positive findings in terms of biochemical test identification and confirmation.

Table (3-2): Saliva level of Tumor Necrosis Factor-alpha in *Viridans streptococci* in oral infected patients and controls. ($P \leq 0.05$)

Groups	Number	Serum Level of TNF- α (pg/ml)			P $\leq$
		Mean $\pm$ S.D.	Minimum	Maximum	
Patients	100	95.05 $\pm$ 25.90	32.0	113.98	0.05
Controls	50	24.11 $\pm$ 1.35	3.57	40.58	

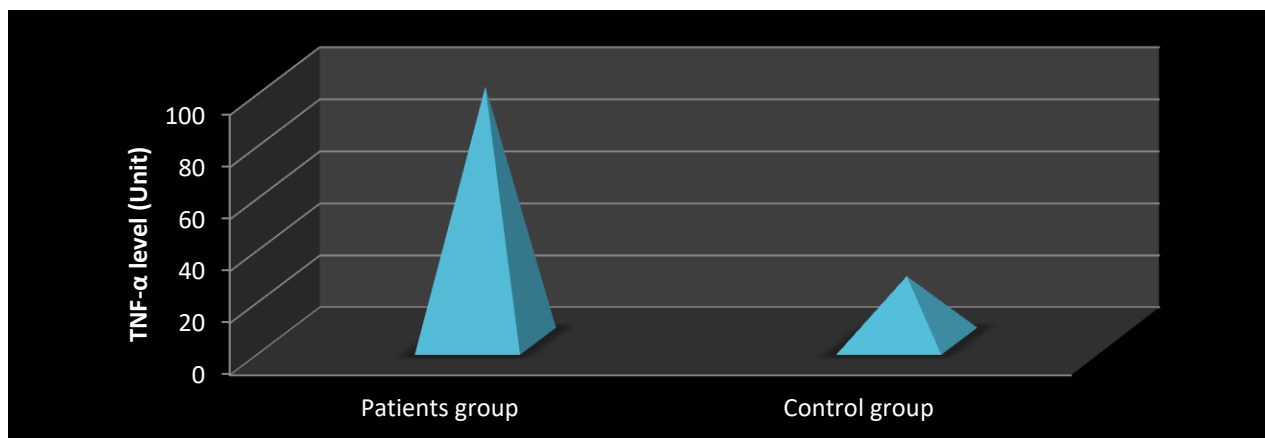


Figure (3-2): Saliva level of Tumor Necrosis Factor-alpha in oral patients infected with *Viridans streptococci* and in controls.

3.4 Results of ABO blood group typing

The table (3-3) and the figure (3) show the numbers and percentages of A, B, AB, and O blood groups for each of the patients and the control individuals. Among the control group ($n = 50$), 10 (20.0%) individuals had blood group A, 17 (34.0%) had blood group B, 5 (10.0%) had blood group AB, and 18 (36.0%) had blood group O. Among patients infected with *Viridans streptococci* ($n = 100$), 18 (18.0%) had blood group A, 24 (24.0%) had

3.3. Results of measuring saliva levels of TNF- α in patients and controls

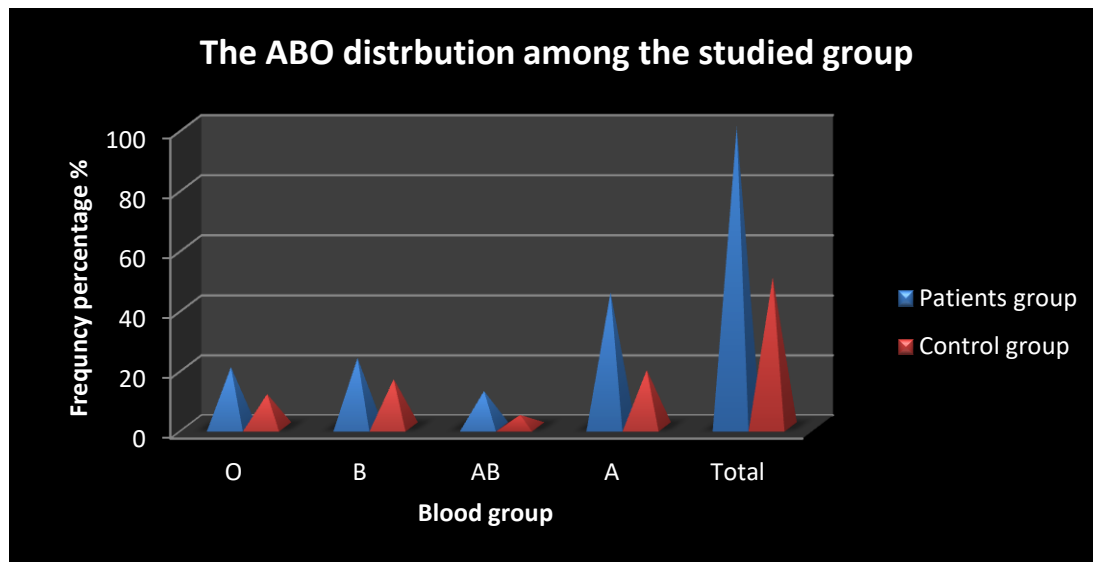
A significantly ($P \leq 0.05$) increased level of TNF- α was observed in saliva of patients infected with *Viridans streptococci* with a mean of (95.05 ± 25.90) pg/ml compared to controls (24.11 ± 1.35) pg/ml (Table 3-2) and figure (3-2).

blood group B, 13 (13.0%) had blood group AB, and 45 (45.0%) had blood group O". As for patients infected with *Viridans streptococci*.

Statistical analysis indicated that there is a significant relationship between O blood groups and infection with *Viridans streptococci*. While those of blood group B ranked second. On the other hand, those with blood group A and blood group AB, coming in third and fourth ranks respectively.

Table (3-3): The ABO distribution between the studied groups.

Blood groups	Patients group No. (%)	Control group No. (%)
A	18 (18.0)%	10 (20.0)%
B	24 (24.0)%	17 (34.0)%
AB	13 (13.0)%	5 (10.0)%
O	45 (45.0)%	18 (36.0)%
Total	100 (100.0)%	50 (100.0)%

**Figure (3-3):** The ABO distribution between the studied groups.

4. Discussion

In this study, the accuracy of VITEK 2 system was shown to be 92% to 98 % for identifying *Viridans streptococci*. The correspondence is at the level of genus, not at the level of species. which agrees with (Ganda and Aryati. 2021)(36) and (Shun, *et al.*2021) (37).

(Ganda and Aryati, 2021) who showed through their research that the accuracy of this device in diagnosing a gram-positive card BioMérieux can identify *Viridans streptococci* with up to 92-98 % but 99.56-100 % for Gram negative bacteria. (36)

A significantly ($P \leq 0.01$) increased level of TNF- α was observed in saliva of patients infected with *Viridans streptococci* infection, that similar results were recorded by (Zhao, *et al.*2021), who reported that infection with *Viridans streptococci* leads to an increase in the level of TNF- α in the patient's saliva due to such deleterious infection. (38)

Stimulation of inflammatory cytokines, such as tumor necrosis factor-alpha (TNF- α) and certain interleukins, via pathogen-associated molecular patterns or modifiers, is a characteristic innate immune response to eliminate microorganisms or limit their spread at the site of entry. (39)

Streptococcus bacteria play a significant role in the development of oral and periodontal diseases, and are central to the progression of tooth decay. They are capable of synthesizing extracellular polysaccharides via the glucose transporter enzyme, which facilitates bacterial adhesion and aggregation, leading to the formation of biofilm and colonization of the oral cavity. Subsequently, the mutant Streptococcus can metabolize carbohydrates to produce acids that act on tooth tissues, demineralizing them and causing decay. (40)

This vital activity of Streptococcus bacteria in oral tissues stimulates the important role of macrophages, promoting the production of

tumor necrosis factor-alpha (TNF- α) and interleukin-1 beta (IL-1 β) by activating the ERK/p38/JNK signaling pathway and nuclear transcription factor kappa B (NF- κ B) via Toll-2 (TLR2) and Toll-4 (TLR4) receptors, respectively (41), NF- κ B then activates a transcriptional response leading to the synthesis of IL-1 β precursors, while a second signaling pathway activates the maturation and secretion of IL-1 β via inflammasomes, dependent on caspase-1 (42), TNF- α initiates a cascade of cytokines that increase vascular permeability, attracting macrophages and neutrophils to the site of infection. (43)

Numerous studies have explored the relationship between blood type and bacterial infections, such as those caused by *Helicobacter pylori* and *Pseudomonas aeruginosa*. (44)

In our research we found patients with blood type O were found to be more frequently associated with *Viridans streptococci* infection compared to control groups, followed by blood type B, then A, and the last one AB. These results were consistent with the researchers' findings (45),

This may be attributed to the antigens of blood type O, which act as receptors for toxins, bacteria, and parasites that are easily colonized or evaded by the body's cleansing mechanisms. Carbohydrate antigens contribute to increased susceptibility to infectious diseases. In particular, for example, the H antigen is expressed in the gastric mucosa and oral tissues of individuals with blood type O, making them more receptive to bacteria. (46).

although these results vary according to populations and geographical distribution between countries, and even among people of the same country, the distributions differ from one region to another. (47)

5. Conclusions

1. Contamination with *viridans streptococci* in oral cavity and tissues is associated with elevated levels of tumor necrosis factor-alpha (TNF- α) in the saliva of

patients, reflecting the inflammatory immune response elicited by *viridans streptococci*

2. Diagnosis of *viridans streptococci* using the VITEK system is more accurate than conventional tests, with an accuracy rate ranging from 92% to 98%.

3. Individuals with blood group O showed a higher frequency of *viridans streptococci* infection compared with other blood groups.

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