Validation of secretory Immunoglobulin A (IgA) for early and efficient diagnosis of *Pseudomonas aeruginosa* lung infection

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ABSTRACT

Pseudomonas aeruginosa is a gram-negative bacterium that is considered to be a major causal organism of nosocomial infection. This study brought data-specific evidence to reveal the efficacy of secretory Immunoglobulin A (IgA) measurement in diagnosing pulmonary *P. aeruginosa* infection and claims its validation as a diagnostic marker. This study has included controls and patients of Pseudomonas and grouped them into four, namely, controls, chronic cases, intermittent cases, and negative group. The last group, that is, the "Negative" group, is the ones who had a history of infection but currently showed negative blood culture. The level of sIgA was quantified in all the patients and the controls and then their status of pulmonary infection was determined by their blood culture. ANOVA and Pearson Chi-Square were employed for showing the association between sIgA and pulmonary infection. The mean value of salivary sIgA has been found the highest in chronic cases followed by Intermittent cases and Negative Infections. The boxplot diagram showed several parameters of sIgA quantification in each group and control. ANOVA and Pearson Chi-square ($P<0.005$) tests showed a significant association between sIgA level in saliva and pulmonary infection of *P. aeruginosa*. The ROC curve was plotted to determine the cut-off value of sIgA (sIgA$\geqq$13.09 U/ml) for efficient clinical diagnosis of pulmonary *P. aeruginosa* infection. The study has validated statistically that quantification of salivary sIgA can be used in clinical practice for early diagnosis of pulmonary infection of *P. aeruginosa*.

Introduction

Pseudomonas aeruginosa is a gram-negative bacterium, which can also be opportunistic, and may result in urinary tract infection, sepsis, keratitis, and most importantly acute and chronic pulmonary infection. *P. aeruginosa* is considered to be a major causal organism of nosocomial infection (1). *P. aeruginosa* is the most common type of bacteria that is present in environments like water and soil. *P. aeruginosa* commonly infects humans apart from other types of pseudomonas. It causes post-operative infections in the lungs, blood, and other body parts. These bacteria are constantly finding ways in becoming resistant to antibiotics, which makes them multi-drug resistant. Patients in the hospitals especially those who are on ventilator support, who have surgical burns or wounds, and those with catheters (2). *P. aeruginosa* is contracted to people in healthcare systems when they get into contact with water or soil that is contaminated with bacteria. The resistant strain of pseudomonas also spreads through contaminated surfaces, equipment, and hands (3).

P. aeruginosa has an inherent resistance to quaternary amines as well as other microbicides which makes it difficult in eradicating the bacteria from the surfaces and equipment of the healthcare units4. The increase in the mortality rate due to infections caused by *P. aeruginosa* is due to the combined action of the weakened host defence mechanism, the resistance of the bacteria to the antibiotics, and the production of extracellular toxins and enzymes by the bacteria (3,4).

In the lung infection caused by *P. aeruginosa,* there is massive recruitment of neutrophils into the airways that are infected, which play an important role in clearing the acute pulmonary infection. *P. aeruginosa* pneumonia is determined by the ability of the body to recruit and kill sufficient numbers of neutrophils in the airways in response to the invasion of *P. aeruginosa* (5). The alveolar macrophages are the first immune cells that encounter *P. aeruginosa*, they can kill bacterial pathogens by internal derangement of the cell as its primary importance is to sense the pathogens. The alveolar macrophages also have an important role in phagocytosis of the dying neutrophils and also initiate repair (6).

P. aeruginosa causes nearly all of the localized infectious diseases that result in fatal bacteremia following burns or surgery. There are instances where pseudomonas aeruginosa is introduced into irrigating solutions or catheters causing urinary tract infections. Most of the patients

with cystic fibrosis are seen to be colonized by *P. aeruginosa* and interestingly these patients have high levels of antibodies circulating in the blood against pseudomonas, which prevents them from having *P. aeruginosa* bacteremia, but most of these patients die due to the localized infections caused due to *P. aeruginosa*. Using the respirators contaminated by *P. aeruginosa* may lead to the development of necrotizing *P. aeruginosa*. It may also cause corneal infections post-operatively (7). It occasionally causes meningitis and endocarditis following a lumbar puncture and cardiac surgery respectively. Gram-negative bacteremia has become increasingly linked to this organism, which currently accounts for 15% of all cases of bacteremia. 50% of the overall mortality is associated with *P. aeruginosa*. Among the different types of infections, some, like infections of the ear and eyes, tend to remain localized while others, such as infections in patients with lymphoma and leukaemia, cause widespread infections. The difference in the spread of the infections is mostly due to the host defence mechanism (7,8).

PCR is one of the methods that help in detecting and identifying *P. aeruginosa*. To synthesize a complementary strand of DNA, primer-mediated enzymatic amplification of DNA is used (9).

As a result of advancement in the polymerase chain reaction, isothermal amplification methods that include polymerase spiral reaction and loop-mediated isothermal amplification have been developed. The isothermal amplification method requires only basic equipment like a heat block with minimal training of the operator and the results are provided within an hour. This method is especially useful for clinical screening when there is a lack of resources (10). Pulse-field gel electrophoresis is a popular method used for large-scale epidemiological investigations as it has discriminatory powers, it separates large molecules of DNA that range between 10 kb to 10 Mb by the application of electricity on a solid matrix (11).

Materials and Methods

Research Subjects

This study has enrolled patients and controls from the Inpatient Department of Internal Medicine and the Outpatient Department, respectively. The study considered those inpatient patients, who were admitted between June 2021 to December 2021. The study subjects were classified into 4 groups, healthy controls, patients with chronic infection of *P. aeruginosa*, patients with intermittent infection and patients, who had an infection of *P. aeruginosa* in the past ($\leqq$12 months) but presently there is no evidence of infection. The positive culture of *P. aeruginosa* is considered evidence of infection.

Inclusion and exclusion criteria

The study employed a set of inclusion and exclusion criteria. The patients, who showed evidence of *P. aeruginosa* infection were to be included in the Chronic cases and Intermittent cases groups. For the "Negative Infection" group, the study considered the history of infection. The patients, who gave consent to undergo culture and cooperate with the whole study process were only included. The age group and socio-economic background were also considered in the inclusion criteria. The patients who had any additional chronic conditions or were immunocom-

promised were excluded from the study. The patients who did not want to give consent for bacterial culture or did not cooperate till the end of the process were excluded.

Collection of Samples

The peripheral blood sample collection was done from 50 subjects (40 patients and 10 controls) and the subject's history was taken. Along with the finding of peripheral blood samples and history, the patients were classified into any one of these three groups, namely, "Chronic cases", "Intermittent cases" and "Negative" groups. The healthy controls were considered from the outpatient department. The blood sample was taken for bacterial culture and brought a conclusion regarding the status of the infection. While the oropharyngeal swab was taken from each subject for quantification of secretory IgA in U/ml.

Quantification of Blood Sample and sIgA

The peripheral blood sample was collected from each subject in 4 ml blood tubes without adding anti-coagulant additives. Then it was centrifuged at the rate of 2500 rpm for 15 minutes. The culture result was considered to be the basis of the infection status for the subject. For sIgA quantification, saliva samples were collected using cotton swabs after keeping the swab in the subject's mouth for 2 minutes. Then this swab was placed in a tube and centrifuged at the rate of 3000 rpm for 12 minutes. This resulted in obtaining pure saliva for sIgA quantification. A 96-well ELISA method was employed for measuring sIgA in saliva and Tetramethyl benzidine was used as substrate. After the reaction continued for 20 minutes, sulphuric acid at the concentration of 1 mol/l was added. The quantification of sIgA was done both in controls and in patients.

Statistical Analysis

The study employed extensive statistical analysis for obtaining several significant results. Firstly, the study showed the base characteristics of the subjects including healthy control. Using SPSS 25, the study has presented a comparative analysis between the mean values of sIgA among each group. For testing association, ANOVA and Pearson Chi-Square were used. The Boxplot diagram has shown several parameters of sIgA in each group. The ROC curve was plotted to show the applicability of sIgA quantification and determine the cut-off value of sIgA in this study.

Ethical Aspects and Consent details

The study and its processes were approved by the ethics committee of the institution. All the subjects who agreed to participate provided written informed consent.

Results

The base characteristics of 50 patients are given below. There are 4 groups including 10 subjects in the Control group, 15 patients in the Chronic cases group, 15 patients in the Intermittent cases group and 10 patients in the Negative infection group (Table 1).

The mean value of secretory IgA in each group of patients was found. The mean value in the Chronic cases group was found to be the highest (498.35±148.21 U/ml) followed by the mean value in the patients of the Intermittent Cases group and Negative Infection group. However,

the difference in the mean value of sIgA of chronic cases and intermittent cases is much higher as compared to the difference between the Intermittent group and Negative Infection. Therefore, it is found that the level of secretory IgA in chronic infection of *P. aeruginosa* is significantly higher as compared to the others in the study (Figure 1).

The study has conducted ANOVA and Pearson Chi-Square for the testing association. The test of association has shown a significant association between the mean value of sIgA (U/ml) with pulmonary infection of *P. aeruginosa*. The values and results of significance for each test of the association are given below (Table 2).

Figure 2 below shows the boxplot diagram of the quantification of sIgA in each group. The measurement of sIgA has been found the highest in the Chronic group. The healthy controls have shown significantly less amount of sIgA. The diagram (Figure 2) shows the range, mean and stan-
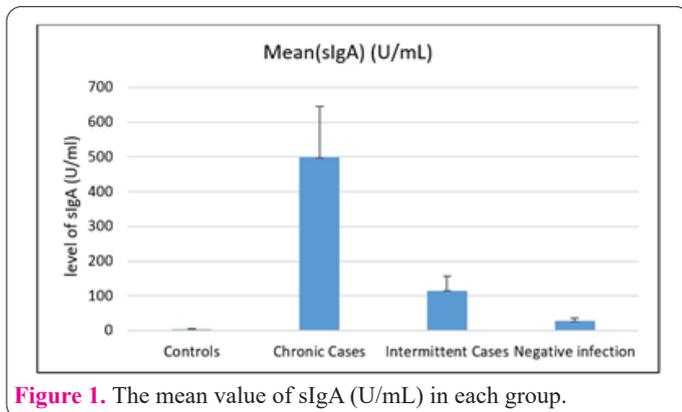

Figure 3. Boxplot diagram showing several parameters of sIgA (U/mL) quantification in each group.

Figure 1. The mean value of sIgA (U/mL) in each group.
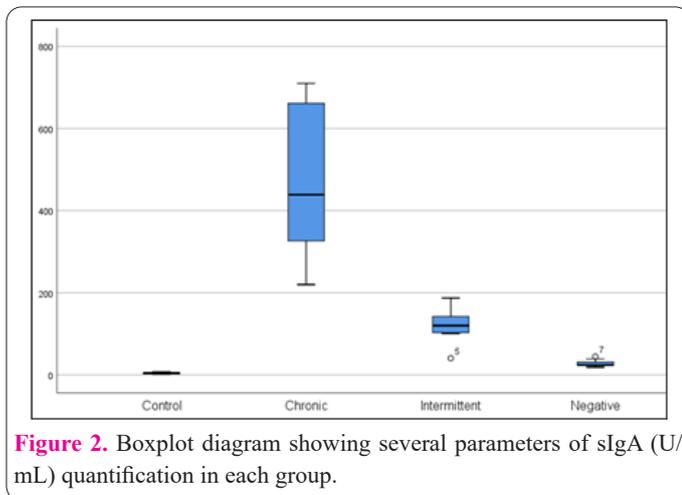
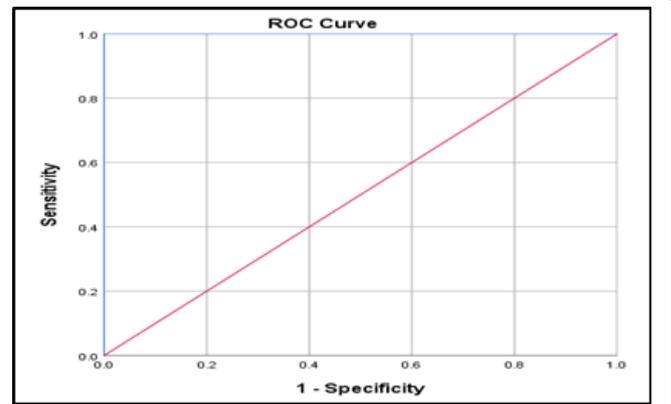**Figure 2.** Boxplot diagram showing several parameters of sIgA (U/mL) quantification in each group.

dard deviation for sIgA measurement for each group of subjects.

The ROC curve reveals a large area under its curve. The ROC curve in Figure 3 below shows that the measurement of sIgA is clinically significant in determining the pulmonary infection of *P. aeruginosa*. Therefore, the secretory IgA can be determined for diagnosing *P. aeruginosa*. The ratio of Sensitivity/Specificity and the subsequent coordinates of the ROC curve shows that the sIgA$\geq$13.09 U/ml can be considered as the cut-off level for diagnosing pulmonary *P. aeruginosa* infection.

Discussion

During the past two decades, *P. aeruginosa* has emerged as an important pathogen causing 10-20% of nosocomial infections. The infections caused by *P. aeruginosa* are most prevalent in patients with burns, acute leukaemia, IV drug addiction, cystic fibrosis, and organ transplants. It is a nasocomical contaminant. Patients, who are hospitalized for extended periods show a high risk of getting contracted the infection. The most serious infections caused by *P. aeruginosa* are endocarditis, pneumonia, endophthalmitis, septicemia, meningitis, and external otitis (12). Penicillins and antipseudomonal aminoglycosides have been known to improve the prognosis of the disease. In treating the infection in patients with neurotrophic disease ticarcillin and carbenicillin have been known to be beneficial. Recently, several new antipseudomonal drugs have been introduced, including penicillins, cephalosporins, and other beta-lactams. These agents may offer novel approaches to treating

Table 1. The base characteristics of subjects included.

	Controls	Chronic Cases	Intermittent Cases	Negative infection	P-value
N	10	15	15	10	-
Gender (M/F)	6/4	8/7	6/9	5/5	-
Mean Age	37.2	37.4	36.53	37	-
SD (Age)	5.78	3.54	3.96	2.90	0.295

Table 2. Findings of tests of association (ANOVA and Pearson Chi-Square).

	df	Value	Significance
ANOVA Regression	1	11.483	.001
Pearson Chi-Square	3	645.000	.000
Likelihood Ratio	3	48.639	.000

df= Degree of freedom

these infections (13-15).

In the year 1908, Paul Ehrlich and Ilya Metchnikoff were awarded the Nobel prize, which led to the discovery of a myriad of mechanisms governing the immune response to microbes. The infectious agent has appeared to be in the form of a planktonic state in various studies. Very little information is known about the response of the immune system to infections based on biofilm. Recent in vitro and in vivo studies show that both the adaptive and innate immune systems respond to biofilms. On the other side to achieve protection against the immune responses the biofilm bacteria have launched several measures that have also been demonstrated to find whether a particular immune response exists to particular biofilm infections. However, as the pathogens are often chronic a situation arises where neither of the host immune response can eliminate the pathogen but instead causes damage to the tissue (16-18).

It is generally said that lethal infections develop due to the invasion of the pathogen in the immunologically compromised host or physiologically stressed patients. Another hypothesis states that the pathogens actively sense the change in the immune response and return response by enhancing the virulent property. It is demonstrated that the interferon-gamma binds to the outer membrane of the *P. aeruginosa* thus resulting in the PA-I lectin the quorum-sensing dependent virulence determinant (19).

P. aeruginosa is a gram-negative bacterium that is versatile and opportunistic in terms of the mechanism of virulence, metabolic potential, and genetics. This versatile nature of the bacteria helps it in responding to varied environmental conditions. The mechanism of gene exchange like conjugation, transduction, and transformation help *P. aeruginosa* in adapting to the changing conditions by adapting new genetic information. Recombinant DNA methods and transposon mutagenesis techniques are recently used to study the virulent factors of *P. aeruginosa* (20). The pathogenesis of *P. aeruginosa* is multifactorial as it produces numerous virulent factors or toxins and also various diseases. The virulent factors of the bacteria help in contributing to several stages of pathogenesis. Polysaccharide slime, pili, and lipopolysaccharide are the surface factors that contribute to the first two stages of pathogenesis. Proteases, toxins like exotoxin A, and hemolysin contribute to the damage of the tissue and dissemination; it also helps in acquiring nutrition in the early stages of the infection that is required for the bacteria. Phospholipase C and alginate production have been shown to have special significance in pulmonary infections like cystic fibrosis (21,22).

The risk factors associated with pseudomonal bacteremia at St Thomas' Hospital, London, between 1969 and 1989 were determined using logistic regression analysis. A linear model for the diagnosis of *P. aeruginosa* bacteremia was constructed using the coefficients of the final logistic model. Another set of patients was validated and detected in the year 1988 to 1991 at Beilinson Medical Center, Petah Tiqva, Israel (23).

Past or present treatment with antibiotics, corticosteroid, and cytotoxic treatment, hospital-acquired infections, detection of the bacteria in the intensive care unit, neutropenia, based on the gender mainly males, foci of the infection are the seven factors that are significant and are independently predictive of pseudomonas infections. The urinary tract with a catheter and post-operative ins-

trumentation is known as the high-risk foci. The female genital tract, bone, meninges, joints, and upper respiratory tract are the low-risk foci. The index divided the patients into three groups with the increase in the prevalence of *P. aeruginosa* are 1%, 7% and 19%, respectively. The study concludes that the use of simple and easy laboratory and clinical data that is known within hours of the detection of the infection in patients helps in differentiating the patients as high or low risk for *P. aeruginosa* bacteremia (24-26).

The study has shown statistical evidence that the quantification of sIgA from saliva can be used to diagnose pulmonary lung infection caused by P. aeruginosa. The chronic condition of P. aeruginosa infection can be best diagnosed by sIgA measurement, while Intermittent cases and past cases which currently show negative culture, can also be detected by sIgA determination. This study has also brought forward the serum level of $sIgA \geqq 13.09$ U/ml should be considered as the cut-off value for diagnosis. The collection of sIgA using a swab and its quantification is significantly easier and more effective in diagnosing P. aeruginosa lung infection. Thus, the determination of the salivary level of sIgA in diagnosing pulmonary infection of *P. aeruginosa* can be considered validated. However, the authors suggested that a similar study should be conducted on larger and variant populations and in different age groups, especially in the pediatric population.

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