<u>Original Research Article</u>

Bacterial Coinfection and Evaluation of Serum Procalcitonin Level as Biomarker for Disease Severity among Admitted COVID Patients

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Abstract

Background: Bacterial coinfections often present in viral respiratory infections and are important causes of morbidity and mortality. The prevalence of bacterial infection in patients infected with SARS-CoV-2 is an important diagnostic criterion. One important diagnostic criterion is PCT values that may be the indication of increased risk of coinfection, thus increasing the mortality rate in critical COVID-19 patients.

Methods: We performed the culture of the Tip of Oxygen pipe, catheter tip, endotracheal tube tip, sputum and blood collected from the confirmed covid positive patients to know the prevalence of acute bacterial coinfection. PCT value were also taken as specific diagnostic criteria in suspected cases of bacterial co-infection.

Result: Microbiological investigations were recorded for 1740 patients and the bacterial Coinfection rate was 5.5%. out of 93 coinfected patient's samples, 37 samples were of blood, 26 of sputum and 30 samples of catheter tip, endotracheal tube tip, pus. most common pathogens were Klebsiella followed by *Pseudomonas, E. coli, Acinetobacter* and *Staphylococcus aureus*. we also observed that increased PCT values for the coinfection higher pct values had high risk of coinfection thus high mortality rate in critical covid19 patients.

Keywords: Bacterial Coinfection, Procalcitonin, Mortality, SARS-CoV-2.

Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is an enveloped RNA β - coronavirus causing severe pneumonia. The SARS-CoV-2 infection has become worldwide public health challenge. The World Health Organization (WHO) named this COVID-19 pneumonia as coronavirus disease 2019 on 11 February 2020. [1] This Coronavirus infection rapidly spread Worldwide and was declared as global pandemic by the World Health Organization on 11 March 2020. 226 countries and Territories around the World have been affected with 505,246,921 confirmed cases and 6,224,814 deaths till April 19,202.

Many researches have proved the relationship of bacterial, fungal coinfection in viral respiratory infection.

Bacterial coinfection with SARS-CoV-2

It was proved by Zhou et al and many research team that in critical cases of viral respiratory infection, bacterial coinfection are usually common. [2] Prof. Zhong and zhang also proved that patients, who were

positive for SARS-CoV were also coinfected by bacteria and fungus. Bacterial coinfection is more common and it complicates the treatment thus increasing the mortality rate. On the other hand, unnecessary prescription of broad-spectrum antibiotics may increase the antibiotic resistance problem. [3] In one study from 38 Michigan hospitals, antibiotics may have been started in COVID-19 patients out of concern, i.e., "just in case." though having low prevalence of confirmed bacterial infection (3.5%).

It was observed that covid -19 patients were suffering from many other complications but the most common is coinfection that increases the severity and mortality. [2] The prevalence of co-infection was present in many covid patients who died of infection, as reported in few papers thus increasing the mortality rate of COVID-19 patients. [4,5]. In admitted Covid patients, initially bacterial co-infection was clinically diagnosed in order to start empiric anti-bacterial therapy as many hospital were short of hands to do culture immediately with admission in hospital. The early recognition and managements of bacterial co-infection is needed in order to decrease the mortality of COVID-19 patient.

Reason of bacterial or fungal coinfection

SARS-CoV-2 infection cause immune system's impairment by damaging B cells, T cells, and NK cells, which may be the main reason for the coinfection. [6] Invasive proceedures like prolonged catheterization, intravenous therapy resulting in secondary infections. Proinflammatory cytokines mainly IL-6 that is responsible for lung injury, increased significantly in COVID-19 patients. [7]

Many studies have shown that due to short of hands during COVID-19 pandemic, excessive antibiotics were prescribed extensively during treatment, and empirical antibiotic were given to 90% of admitted patients. Klebsiella, *Acinetobacter baumannii, Escherichia coli, Pseudomonas aeruginosa,* and *Enterococcus spp* are important bacteria causing coinfection. [8]

The diagnosis of bacterial coinfection

the diagnosis of coinfection in COVID-19 patients, is very difficult only on the basis of clinical and radiological investigations as experimented by Azoulay et al. 2020. In order to start empiric anti-bacterial therapy, clinical diagnosis as well as other diagnostic criteria needed beside culture. It was reported in many articles that calcitonin detection may also be included as one of the factors in the diagnosis of coinfection. [9] It was proved that there will be the massive production and release of parathyroid- derived calcitonin during bacterial infection due to increase in interleukin, tumor necrosis factor (TNF), and IL-6. Thus Procalcitonin (PCT) is also an important biomarker and can be used as diagnosis of coinfection with other laboratory findings. [10]

PCT is normally less than 0.05ng/ml in healthy individuals. Elevated level of PCT assess the degree of severity, Prognosis of the outcome, benefits from antibiotic treatment to patients and monitoring of antibiotic therapy within the measuring range. However normal level donot exclude infection but it should be corelated clinically.

In one of the study in China it was analysed that that PCT was <0.5 ng/mL in cases with no complications. Most COVID-19 patients even had PCT values < 0.25 ng/mL or even <0.1 ng/ml. In cases of higher severity of disease and infection control, PCT was found to be higher (> 0.5 ng/mL). [11] Previous reports of viral epidemics also proved that PCT is usually low (<0.1 - < 0.5 ng/mL) in admitted patients with pure viral infection.

PCT value can be considered in prescribing antimicrobials in COVID-19 patients. The patients having insignificant PCT value can avoid antimicrobial use till further report.

Material and methods

In the present study, along with the nasopharyngeal swabs, we also collected sputum and blood samples, the Tip of Oxygen pipe, catheter tip, endotracheal tube tip, from the Covid -19 patients in Triage area to know the prevalence of acute bacterial coinfection. For the early detection and differential diagnosis of clinically relevant bacterial infection Procalcitonin test is done in COVID positive patients. Out of 4295 Covid patients, total 1740 (40.5%) clinically significant COVID-19- patient samples were sent to microbiology labs for investigation. We collected 1740 samples of sputum and blood samples, the Tip of Oxygen pipe, catheter tip , endotracheal tube tip, from the confirmed COVID-19 patients and sent to the microbiology lab for investigation. We used VITROS 5600 integrated system for the quantitative measurement of Procalcitonin (PCT) in human serum. Measuring (Reportable) range is 0.030 -100 µg/L

Result

We performed the culture of the Tip of Oxygen pipe, catheter tip, endotracheal tube tip, sputum and blood collected from the confirmed covid positive patients to know the prevalence of acute bacterial coinfection.. The study conducted from April 2019 to April 16, 2020. Total 4295 Covid patients were admitted in Era's medical College. All samples were collected from Covid patients admitted in Covid wards including ICU, HDU, CCU. we also checked the PCT value as calcitonin may be the diagnostic criteria for coinfection of bacteria in critically ill patients, leading to the various complications.

During 1st wave Total no of covid patients admitted in ELMCH from 4th july till 20 Feb were 3240. During 2nd wave total no of Covid patients admitted in ELMCH from 25th March till 12th July were 1055.

Microbiological investigations were recorded for 1740 patients, the bacterial Coinfection rate was recorded in 93 patients (5.5%). Among these 31(34%) were in Covid isolation ward and rest 62 (66%) admitted in Intensive care units and some of them are on ventilators. Severely affected patients admitted in intensive care unit had higher rates of coinfection. out of 93 coinfected patients' samples, 37 samples were of blood, 26 of sputum and 30 samples of catheter tip, endotracheal tube tip, oxygen pipe tip, pus.

Enterobacteriaceae (*E. coli, Klebsiella*) and Enterococcus followed by Acinetobacter were the common pathogens causing Bloodstream infections. Respiratory co-infections having Enterobacteriaceae most and among these common pathogens were Klebsiella and Pseudomonas. In ET and Tip cultures also Klebsiella were most common in Enterobacteriacae followed by *E. coli, Pseudomonas, Acinetobacter* and *Staphylococcus aureus*.

Table 1: Percentage of coinfection in samples of admitted covid patients

S. No	Microorganisms	Blood	Sputum	Tips /pus	Total (%)
1	*E. coli*	8	3	4	15 (16%)
2	*Klebsiella*	3	12	18	33 (35%)
3	*Pseudomonas*	-	7	4	11 (11.8%)
4	*Enterococcus*	4	1	-	5 (5.37%)
5	*Acenitobacter*	2	-	2	4 (4.3%)
6	*S. aureus*	-	-	1	1 (1%)
7	*CONS*	20	3	1	24 (25.8%)
	Total	37	26	30	93 (5.5%)

CONS : coagulase negative Staphylococcus ; Tips: catheter tip, endotracheal tube tip, oxygen pipe tip. CONS were grown in20 samples of blood culture in ICU patients.

Table 2: Age wise and Gender wise distribution

S. No	Samples	Age		Sex	
		> =50yrs	< 50yrs	Males	Females
1	Blood	23	14	24	13
2	Sputum	19	7	17	9
3	Catheter tip, oxygen pipe tip, ET, Pus	22	8	22	8
	Total	64	29	63	30

Out of 93 coinfected patients, 64 were >=50 yrs and rest 29 were <50 yrs of age. In case of gender wise distribution, 63 male patients samples and 30 female patients samples were coinfected among 93 samples

The rate of severe bacterial coinfection is significantly higher in male patients and patients having >=50 years of age.

Beside culture we also Monitor PCT to detect secondary infections and its severity. Patients with less severe symptoms had PCT values <0.1 ng/mL.1-6. All severely infected patients admitted in ICU or HDU had PCT value ranging from 0.14ng/ml to 35.6 ng/ml having bacterial Coinfection. Even Blood culture samples having CONS, the PCT values are greater than reportable range.

Table 3:   PCT values (range) in admitted Covid patients

S. No	Samples	PCT value
1	Blood	0.14 - 35.4
2	Sputum	0.07 - 2.01
3	Catheter tip, ET, Pus	0.07 - 35.6

In blood samples, the PCT value of 3 patients are 0.14,0.14,0.18 rest having range from 0.6 to 35.4. In sputum samples the PCT value of 2 patients are low ie: 0.7 and other ranging from 0.51 to 2.01. In other samples PCT value of 4 patients are 0.07, 0.14, 0.14, 0.18 and rest ranging from 0.24 to 35.4.

Discussion

Among various pathogens coinfection, particularly bacterial coinfection is the the most common type of infection in SARS-CoV patients. Previous studies had shown the bacterial co-infection occurred between 15 to 28% of cases in critically ill COVID-19 patients. [4,5] In our study Microbiological investigations were recorded for 1740 patients and the bacterial coinfection rate was 5.5%. Among these 31 were in Covid isolation ward and rest 62 (66%) admitted in Intensive care units. In one of the studies by Zhang et al. 2020. [12] the bacterial coinfection rate is 7.7% among 221 SARS-CoV-2 pneumonia patients. In the above study 25.5% bacterial coinfection present in the severely affected patients having pneumonia whereas less severe patients having 0.8% coinfection rate

Rawson et al [8] reported 8% patients with fungal and bacterial coinfection during hospital admission. Bacterial coinfection rate in severely affected patients is higher in our study due to higher number of patients in our microbiology record. Huttner et al. 2020 from Italy found 11% coinfection rate with other bacteria and fungi among the 16,654 patients having severe illness and some of them died of SARS-CoV-2 infection. [13] Chen et al. (2020) in Wuhan reported *Klebsiella pneumoniae* and *Acinetobacter baumannii* coinfection among the 99 SARS-CoV-2 pneumonia patients. [14] In our study also most common pathogens were Klebsiella followed by Pseudomonas, *E. coli, Acinetobacter* and *S. aureus*. Rawson et also reported same bacterial profile as coinfection in admitted COVID patients. [8]

We reported CO NS in blood culture in patients admitted in intensive care units. Though Interpretation of blood cultures that are positive for CONS is often doubtful but Coagulase negative staphylococci are increasingly recognized as leading pathogens in bacteremia, with incidence peaking in intensive care units as reported by many researchers. [15,16]

However, it is proved by Garazzino and Bengoechea 2020 that the bacterial co infection rate with SARS - CoV-2 infection increases the severity of the disease , and thus increasing the mortality.

As previously described by Shuetz and Mueller B, we also observed that increased PCT values usually associated with an increased risk of coinfection thus increasing the cure time and mortality rate in critically ill covid19 patients. [9,10] Our findings were also indicating same results that higher PCT values were associated with high rate of bacterial coinfection and thus increasing mortality rate in the SARS-CoV-2 pneumonia . Before the COVID-19 pandemic guidelines, antibiotics were recommended for patients with higher PCT values and in patients with bacterial coinfection.

Conclusion

Viral infections with bacterial coinfection have a worse outcome. It complicates the treatment and may increase the morbidity and mortality rate as was seen in admitted Covid patients. On the other hand, prescribing of broad-spectrum antibiotics when not needed can increase the antibiotic resistance problem. which is one of the major global health problems. So, the early recognition and managements of bacterial co-infection is needed in order to decrease the mortality of COVID-19 patient. unnecessary use of antibiotics in viral infections with low PCT value should be avoided and therefore empirical treatment should be recommended.

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