

Short Communication

Assessment of Knowledge among the Paramedical Staff on the Collection and Processing of Blood Samples for IL-6 Assay in COVID-19 Patients

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A B S T R A C T

Background: While laboratory investigations play a major role in guiding therapeutics in COVID-19, the knowledge of the COVID-19 warriors about the collection and processing of laboratory samples has been challenging. It has been noticed that a delay in performing interleukin-6 (IL-6) assay leads to a wrong interpretation of the results.

Aim: To assess the knowledge of paramedical staff on the collection and processing of blood samples from COVID-19 patients with a special reference to IL-6.

Materials and Methods: A questionnaire involving qualitative and quantitative data was prepared to assess the knowledge of the paramedical staff who were involved in the collection, transport and processing of blood samples. The survey was conducted among 200 paramedical staff and the participants were interviewed through online Google Forms. Cronbach's alpha was used to test the internal consistency of the questionnaire. Frequencies and percentages of items were calculated to assess the knowledge of participants about the collection, transport and processing of biochemical parameters.

Results: More than 30% of the paramedical staff involved in the collection, transport and processing of biochemical samples lacked awareness that a delay in transport and processing would cause false results.

Conclusion: The study participants were given awareness regarding the collection, transport and processing procedures while collecting samples for IL-6 to avoid false interpretation of the results.

Keywords: Questionnaire, IL-6 Assay, Paramedical Staff, COVID-19 Patients

Introduction

In COVID-19 patients, altered biomarker levels are useful to assess the progression of the disease and to categorise them as severe and/ or fatal clinical conditions.¹ Among the biochemical and immunological markers, interleukin-6 (IL-6) levels in COVID-19 positive patients have been considered an element of risk for the severity of the disease, inflammatory storm and rapid pulmonary invasion.² IL-6, a pleiotropic cytokine, which acts as an inflammatory and pro-inflammatory mediator, has a crucial role in B and T cell differentiation, acute phase responses, fever induction, angiogenesis, and iron and lipid metabolism.^{3,4} IL-6 produced at the infection site is released into the blood circulation and delivered to the whole body, resulting in an immediate host immune response.⁴ Several studies suggest IL-6 as a reliable predictor for disease progression and a relevant tool for prognostic evaluation.⁵

Despite the pivotal role of laboratory investigations to assist therapeutics, the awareness among the COVID-19 warriors on sample collection and processing has been challenging. Data have shown that a delay in performing the IL-6 assay leads to wrong interpretation of the test results. Hence, we aimed to assess the awareness among paramedical staff on the collection and processing of blood samples in COVID-19 patients with a special reference to IL-6 assay.

Materials and Methods

The present study was conducted from August 2021-November 2021 after obtaining prior approval from Institutional Ethical Committee (VMKVMC&H/IEC/21/156 Dated 30/7/2021) A questionnaire involving qualitative and quantitative data was prepared to evaluate the knowledge of the paramedical staff at Vinayaka Mission's Kirupananda Variyar Medical College & Hospitals, Salem. The paramedical staff who were involved in the collection, transport and processing of blood samples were included in the study. Clinicians were excluded from the study. (Table 1). A questionnaire-based survey was conducted on 200 COVID-19 warriors, and the participants were interviewed through online Google Forms. Demographic information, such as age, gender, educational qualification and job experience, was obtained from each participant. Knowledge on selection of appropriate blood collection tube, time taken for transport, turnaround time, method of testing, delay in sample processing and wrong interpretation of IL-6 assay results were assessed among paramedical staff.

Statistical Analysis

Cronbach's alpha was used to test the internal consistency of the data. Frequency distribution and percentage for each item were calculated for the knowledge about the collection, transport and processing of blood samples for the assay of IL-6.

Table 1. The Study Questionnaire Used to Assess the Knowledge of Paramedical Staff on IL-6 Assay

S.No.	Questionnaire			
1.	Which colour-capped tube is most suitable for the collection of blood samples for biochemical parameters?			
	a. Red	b. Lavender	c. Light blue	d. Grey
2.	Which colour-capped tube is appropriate for the collection of blood samples for testing IL-6?			
	a. Red	b. Lavender	c. Light blue	d. Grey
3.	What is the average time taken to transport samples from the ward to the clinical laboratory?			
	a. 2 hours	b. 4 hours	c. 6 hours	d. 12 hours
4.	Is there any delay in the transport of samples from the ward to the clinical laboratory?			
	a. Yes		b. No	
5.	What is the maximum acceptable time frame between sample collection and testing of blood samples for biochemical assays in your laboratory?			
	a. 6 hours	b. 12 hours	c.18 hours	d. 1day
6.	What is the maximum acceptable duration between sample collection and testing of IL-6 in your laboratory?			
	a. 2 hours	b. 4 hours	c. 6 hours	d. 12 hours
7.	Within how many hours does IL-6 need to be measured in blood samples?			
	a. 1 hour	b. 2 hours	c. 3 hours	d. 4 hours
8.	Are you aware that a delay in performing IL6 in blood samples leads to false positive results?			
	a. Yes		b. No	
9.	What is the method used to measure IL-6?			
	a. Immunoassay	b. PCR	c. Kinetic assay	d. Endpoint method
10.	What is the normal level of IL-6 in serum?			
	a. 1.8 pg/mL	b. 1.8 ng/mL	c. 1.8 mg/mL	d. 1.8 µg/mL

Results

Assessment data on the knowledge of appropriate sample collection containers showed that 83.5% (167/200) of participants mentioned the red colour-capped tube as the appropriate tube to be used for the collection of blood samples for biochemical parameters, 11% (22/200) selected the lavender-capped blood collection tube, and 5.5% (11/200) selected light blue tube. About 77.5% (155/200) of participants answered that samples for IL-6 assay need to be collected in the red-colour capped tube, whereas 11% (22/200) mentioned the light blue tube, 6% (12/200) mentioned the lavender tube, and 5.5% (11/200) answered the grey tube.

Regarding data on knowledge of sample transport, 66.5% (133/200) of the participants answered that the samples were transported to the laboratory from the site of collection within two hours, and 33.5% (67/200) mentioned four hours. About 44.5% (89/200) of the participants remarked on the delay in the transport of samples from the ward to the clinical laboratory.

Responses for turnaround time of samples for biochemical parameters have shown that 50% (100/200) of the participants answered six hours, 22% (44/200) mentioned

one day, 11% (22/200) responded 12 hours, and 17% (34/200) answered 18 hours. About 55.5% (111/200) of the participants answered that the turnaround time for IL-6 was two hours, 16.5% (33/200) mentioned six hours, 9% (18/200) answered 12 hours, and 19% (38/200) of participants mentioned four hours.

About 61% (122/200) of participants responded that IL-6 should be measured in blood samples within two hours, 11% (22/200) mentioned three hours, 17% (34/200) answered four hours, and 11% (22/200) mentioned one hour. (Figure 1)

Data on awareness regarding the delay in analysing levels of IL-6 in blood samples leading to false positive results showed that 66.5% (133/200) of participants were aware of it, and 33.5% (67/200) lacked awareness of the same.

About 44.5% (89/200) of the participants remarked that the method used to measure IL-6 was immunoassay, 22% (44/200) mentioned PCR, 11% (22/200) selected kinetic assay, and 22.5% (45/200) mentioned end point method.

About 55.5% (111/200) of respondents answered that the normal level of IL-6 in serum was 1.8 pg/mL, 28% (56/200) responded 1.8 mg/mL, and 16.5% (33/200) of participants mentioned 1.8 ng/mL.

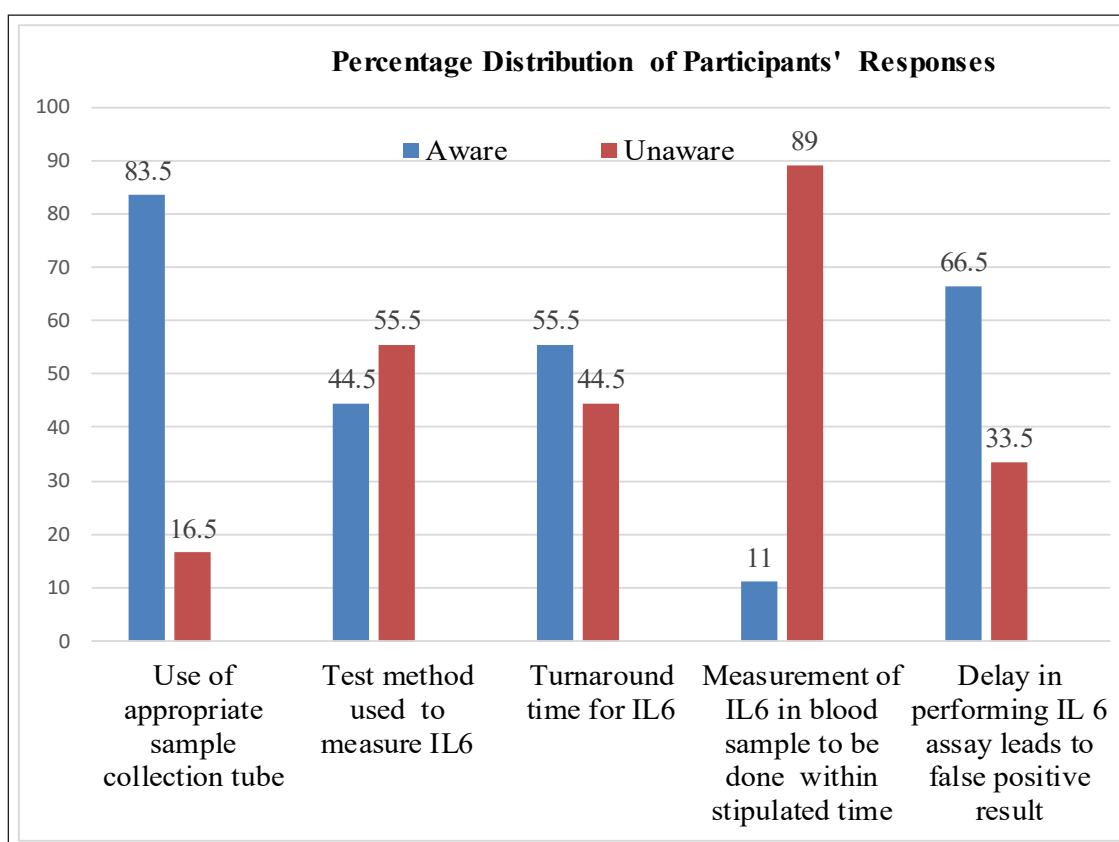


Figure 1. Percentage distribution of participants response on IL-6 Assay

More than 80% of participants in the study were aware of the appropriate sample collection container for the IL-6 assay. However, more than 80% of participants were not aware that blood samples for the IL-6 assay should be processed within one hour after collection to avoid errors in test results. More than 40% of paramedical staff lacked awareness about the turnaround time for the IL-6 assay.

Discussion

The majority of the participants (83.5%) were aware that the blood samples for the analysis of biochemical parameters/ biochemistry analytes should be collected in clot-activated tubes.

In our study, 77.5% of the participants had responded that the blood sample for the estimation of IL-6 was drawn in clot-activated tubes. More than 80% of participants were aware of the appropriate sample collection containers for IL-6 assay. Though many kit protocols suggest that serum may be used for assay of IL-6, studies suggest heparinised plasma or ethylenediaminetetraacetic acid (EDTA) samples may be used for testing IL-6. This is due to the phenomenon of bio-incompatibility. In recognition of a foreign surface by the cellular components of blood, activation of complement and coagulation pathways by the plastic surface occurs, which leads to the activation of platelets and other immune cells.^{6,7} Hence, plastic tube coating with heparin/ EDTA can effectively decrease cytokine release and complement activation.^{6,8}

Among study participants, 33.5% lacked awareness that a delay in performing IL-6 assay can result in false positive test results. More than 80% of study participants lacked awareness that blood samples for IL-6 assay need to be processed immediately to avoid test result errors. About 61% had responded that IL-6 assay has to be performed within two hours. It has been shown that in healthy persons, serum IL-6 remains stable for the first four hours at room temperature and enhanced IL-6 levels was observed in samples with long-term storage condition and temperature.^{9,10} Longer post-collection processing delays significantly influenced IL-6 levels in trauma patients when compared to healthy control groups.¹¹ Based on the study report by Srivatsan et al., separated serum or heparinised plasma kept at 2–8 °C can maintain the IL-6 levels stable for up to 24 hours. If refrigeration is unavailable, levels of IL-6 in separated serum or plasma should be measured within 30 minutes.¹²

A significant decline in the levels of IL-6 in response to a delay in the processing of EDTA plasma has been reported.^{13,14} These studies suggest a degradation of IL-6 in unprocessed clinical specimens, yet when the separation of plasma is not delayed, the cytokines remain stable and can be further used for analysis in epidemiological studies.

Experts from CME India learning point on a discussion on IL-6 levels in COVID-19 patients concluded that to eliminate preanalytical errors, after blood sample collection in an EDTA-coated tube, it should be centrifuged within 30 minutes, and the separated plasma should be stored for analysis, if not tested immediately. If the plasma is separated after 30 minutes, it may cause analytical error in the IL-6 test. If samples are collected in plain red-capped tubes, and are allowed to clot, and the serum is not separated immediately, the clotting process causes rapid release of IL-6 and results in falsely high results. The extent of false increase is in proportion to the amount of time it remains in contact with the clotted cells, varying from 3–4 times to 100 times or more. They also suggest that EDTA keeps the level more stable for 24 hours.¹⁵

Studies have shown that pre-analytical test variables such as blood collection tubes, specimen storage temperature, duration, plasma or serum separation, and pretreatment steps can influence specimen quality and test results.^{16,17} Inconsistent laboratory test results can occur if pre-analytical procedures are not standardised. This will limit the application prospects of the assay for clinical judgement. Hence, the participants were given awareness that the sample should be transported immediately and the test should be performed within four hours. If there is a delay in performing the test, the sample may be immediately centrifuged within 30 minutes and stored at 4 °C.

Conclusion

Preanalytical error has shown a great variation in the levels of IL-6 in COVID-19. It is necessary to create awareness on the collection, transport and processing of blood samples among the paramedical staff and COVID-19 warriors for the betterment of the patients. Hence, the study participants were given awareness regarding the collection, transport and processing procedures while collecting samples for IL-6 analysis to avoid preanalytical errors and thus to prevent false interpretation of the results.

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Declaration of Generative AI and AI-Assisted Technologies in the Writing Process:None

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