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Original Research Article

A Study on Effect of Storage Time on Different Sample Types in Measuring IL-6 in a Hospital Based Setup

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Conflict of interest: Nil

Abstract:

Introduction: Interleukin-6 (IL-6), a pro-inflammatory cytokine, concentration increases several fold during inflammatory conditions, making it a biomarker in diagnosis, therapy and prognosis in various diseases. However; several pre-analytical factors may affect the IL-6 assay. In this study, we aim to investigate the effect of storage time in different sample types (serum/plasma) on measurable IL-6 in Covid-19 cases.

Materials & Method: Serum and Plasma (EDTA) samples were collected from 30 Covid-19 cases. The samples were divided into two groups. They were kept at 22–26°C (Lab room temperature). IL-6 was assayed by the Immunoturbidometric Immunoassay method on Vitros 5600 analyser of Orthoclincal Diagnostics. The first group was assayed within 1 hr of collection and the next group was assayed 2 hrs after assay of the first sample. The change in IL-6 levels in both groups for serum and plasma was analysed.

Results & Conclusion: At the baseline, the paired plasma and serum IL-6 values had good correlation without any statistically significant difference ($p>0.05$). With the increase in storage time, a more pronounced (highly significant statistically $p<0.0001$) rise in un-separated serum IL-6 values were observed. But, the plasma values remained stable (no statistically significant difference, $p>0.05$) over the same storage time. We can conclude that samples types and storage time may affect the IL-6 levels. Hence, plasma samples (EDTA) should be considered to ensure the accuracy and stability of IL-6 values.

Keywords: Interleukin-6, Sample Types, Storage Time.

Introduction

Interleukin-6 (IL-6) is an important cytokine that plays a crucial role in many biological processes within the human body. It is involved in regulating immune responses, inflammation, and hematopoiesis. IL-6 is produced by a variety of cells, including fibroblasts, T cells, B cells, and macrophages.[1,2]

IL-6 is involved in a number of physiological and pathological processes, including the acute-phase response to infection and tissue damage, the development and progression of cancer, and the regulation of metabolic functions such as glucose and lipid metabolism.[3,4] It is also involved in the pathogenesis of many inflammatory and autoimmune diseases, including rheumatoid arthritis, multiple sclerosis, and systemic lupus erythematosus.[5] Furthermore, IL-6 plays a key role in the immune response to viral and bacterial infections, as it stimulates the production of other cytokines and immune cells that help to fight off the pathogen. In summary, IL-6 is a highly important cytokine that is involved in many critical biological pathways within the body.[6]

Elevated levels of IL-6 have been linked with the severity of several diseases, including inflammatory and autoimmune diseases, as well as some cancers. High levels of IL-6 have been linked with increased mortality rates in sepsis patients.[7] In lung cancer, IL-6 has been shown to promote tumor growth and metastasis, and high levels of IL-6 have been associated with a poor prognosis.[8] Similarly, in COVID-19 patients, elevated levels of IL-6 have been linked with a more severe disease course and poorer outcomes. This is because IL-6 is involved in the body's inflammatory response to

the virus, and excessive inflammation can lead to tissue damage and organ failure.[9]

As the levels of IL-6 are associated with medical diagnosis, therapy and prognosis, monitoring the change in IL-6 levels may reflect the progression or regression of diseases. Nowadays, remarkable advances in instrument technology have improved the analytical accuracy of IL-6 enormously. However, lack of a standardized handling procedure in analysis influence the biomarker role of a IL-6. Major errors of the entire diagnostic process are generated within the pre-analytical phases.[10,11]

Pre-analytical variables for the test include blood collection tubes, specimen storage temperature and time, plasma or serum separation, and pre-treatment steps. [12,13] The half-life of IL-6 is short, so the degradation and production may occur simultaneously during storage under un-separated condition. In addition, coagulation processes affect inflammatory activity, resulting in up-regulation of pro-inflammatory cytokines in vitro. Usually, the pre-analytical periods are varied for many causes. The immune responses, which are affected by environmental temperature and storage time, are still on-going during the period.[14]

IL-6 is assayed by the Immunoturbidometric Immunoassay method on Vitros 5600 analyser of Orthoclincal Diagnostics in our hospital. However, the change of IL-6 under un-separated condition is poorly understood. Therefore, we examined the effects on concentrations of IL-6 in serum or plasma under different storage time in the present study. This study will help in choosing the ideal sample type (serum/plasma) and thereby carry out the correct measurement of this specific marker in clinical laboratory.

Materials & Method

Serum and EDTA plasma samples were collected from 30 RTPCR confirmed Covid-19 cases. IL-6 was one of the monitoring laboratory tests to be done specially in deteriorating Covid-19 cases as per AIIMS/ ICMR-COVID-19 National Task Force/ Joint Monitoring Group (Dte.GHS) Ministry of Health & Family Welfare, Government of India - Clinical guidance for management of adult covid-19 patients dated 17th MAY, 2021. [15]

The samples were immediately transported to laboratory after collection. The Serum and Plasma samples were divided into two groups. The samples were kept at 22 – 26 °C (Lab room temperature). The first group was assayed within 1 hr of collection and the next group was assayed 2 hrs after assay of the first sample. Serum and Plasma of same case was assayed at the same time. IL 6 was assayed through the Immunoturbidometric Immunoassay method on Vitros 5600 fully automated analyser of Orthoclincal Diagnostics. The change in IL-6 levels in both groups for serum and plasma was analysed. The values obtained were statistically evaluated.

Results & Observation

At the baseline, the paired plasma and serum IL-6 values had good correlation without any statistically significant difference ($p>0.05$). With the increase in storage time, a more pronounced (highly significant statistically $p<0.0001$) rise in un-separated serum IL-6 values were observed. But, the plasma values remained stable (no statistically significant difference, $p>0.05$) over the same storage time.

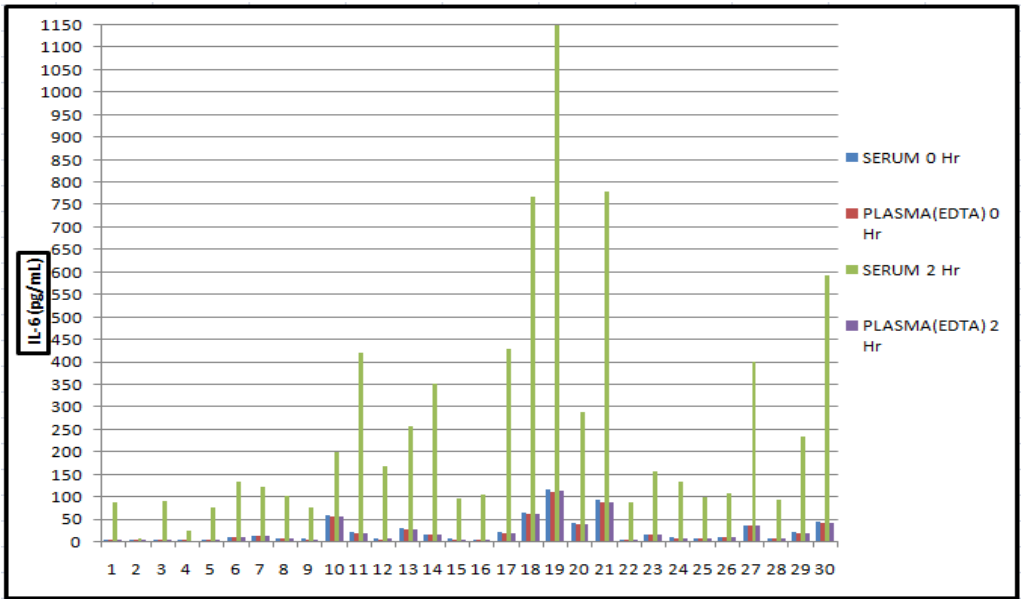

Figure 1: The 0 hour and 2 hour paired Serum and Plasma values

Table 1: Comparison between Serum 0 hour and Plasma (EDTA) 0 hour assay

PARAMETER	SERUM 0Hr (N=30) MEAN ± S.D.	PLASMA[EDTA] 0Hr (N=30) MEAN ± S.D.	COMPARISON
IL-6 (pg/mL)	23.76 ± 27.68	22.14 ± 26.62	p > 0.05 NOT SIGNIFICANT

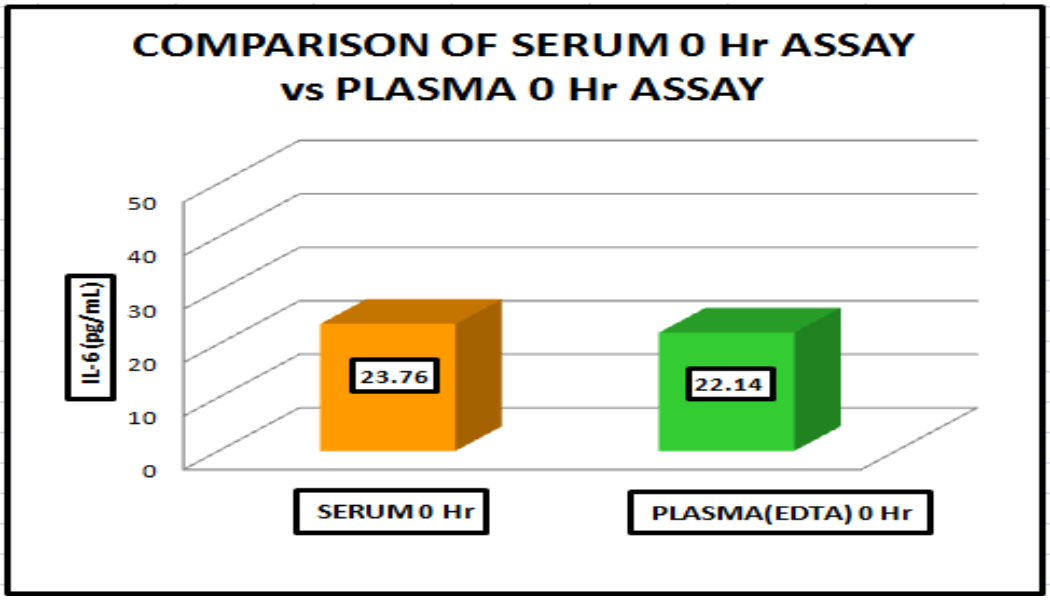

Figure 2: Comparison between Serum 0 hour and Serum 2 hour assay

Table 2: Comparison between Serum 0 hour and Serum 2 hour assay

PARAMETER	SERUM 0Hr (N=30) MEAN ± S.D.	SERUM 2Hr (N=30) MEAN ± S.D.	COMPARISON
IL-6 (pg/mL)	23.76 ± 27.68	254.75 ± 263.44	p < 0.0001 HIGHLY SIGNIFICANT

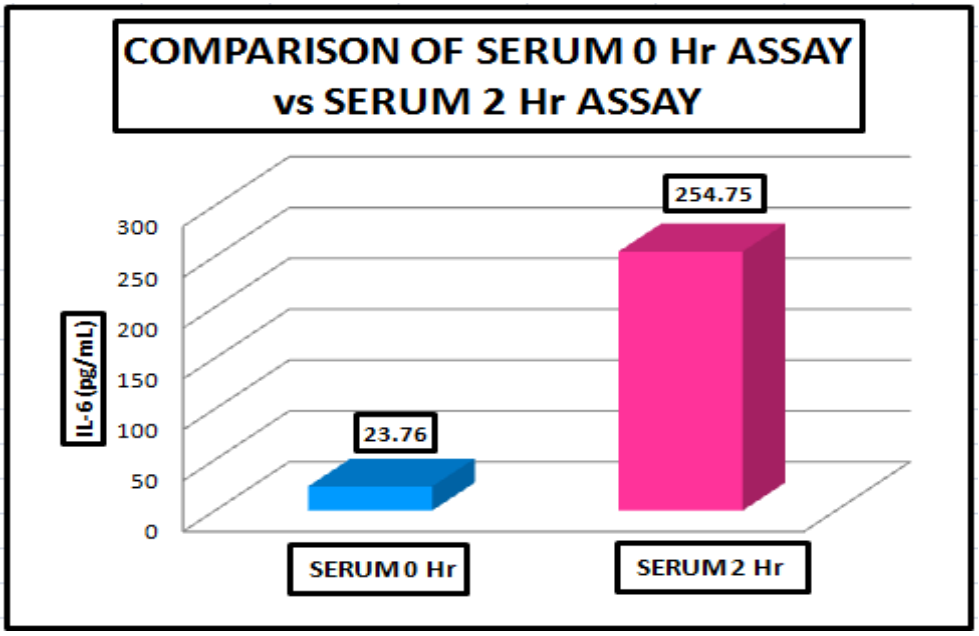

Figure 3: Comparison between Plasma (EDTA) 0 hour and Plasma (EDTA) 2 hour assay

Table 3: Comparison between Plasma (EDTA) 0 hour and Plasma(EDTA) 2 hour assay

PARAMETER	PLASMA[EDTA] 0Hr (N=30) MEAN ± S.D.	PLASMA[EDTA] 2Hr (N=30) MEAN ± S.D.	COMPARISON
IL-6 (pg/mL)	22.14 ± 26.62	22.20 ± 26.71	p > 0.05 NOT SIGNIFICANT

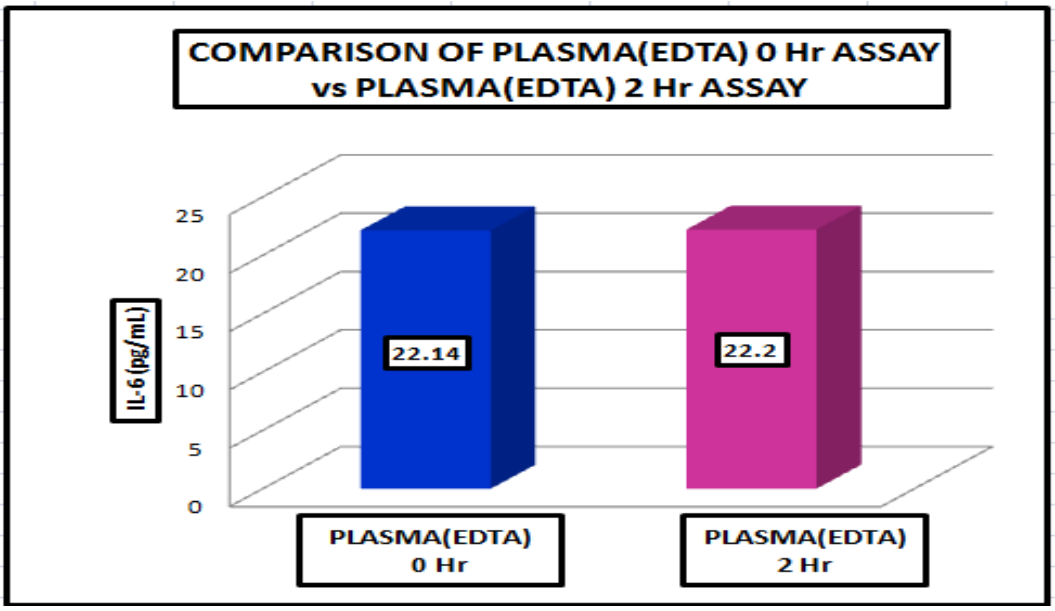

Figure 4: Comparison between Serum 2 hour and Plasma (EDTA) 2 hour assay

Table 4: Comparison between Serum 2 hour and Plasma (EDTA) 2 hour assay

PARAMETER	PLASMA[EDTA] 2Hr (N=30) MEAN ± S.D.	SERUM 2Hr (N=30) MEAN ± S.D.	COMPARISON
IL-6 (pg/mL)	22.20 ± 26.71	254.75 ± 263.44	p < 0.0001 HIGHLY SIGNIFICANT

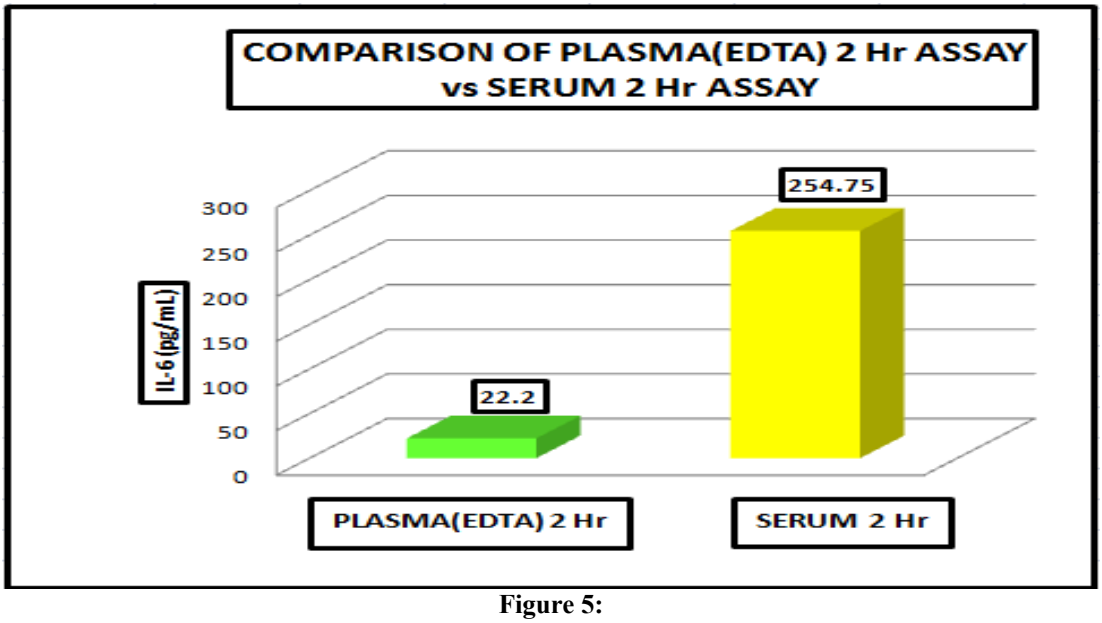

Figure 5:

Discussion

In the present study we found that there was an extremely significant rise in the un-separated serum IL-6 levels when stored at lab room temperature whereas the plasma IL-6 levels remained stable under the same conditions.

The effect of EDTA is to prevent the blood from clotting in vitro, which is distinct from the coagulant. Through sequestering the calcium ions, EDTA stabilizes the whole blood in fluid form. Our study on IL-6 is consistent with those studies that reported that EDTA can maintain the stability of cytokines.[16-18] There is a dynamic balance between the degradation and production of cytokines in vitro. On the one hand, the half-life of cytokines is relatively short, and on the other hand, various stimuli lead to the continuous secretion of cytokines by the blood cells.[19] Coagulation has been confirmed to be one of the factors that can induce cytokine release.

There is an extensive cross talk between hemostasis and inflammation. Inflammation can be modulated by the components of coagulation pathway. Several studies have demonstrated that FVIIa, FXa, and thrombin can active blood cells to produce IL-6.[20,21]

During the blood drawn in serum tubes, the initiation of coagulation pathways is accompanied by the activation of blood cells.[21] We hypothesize that this is the major factor in IL-6 elevation in serum samples on storage.

The main limitation of this study was its small sample size.

The future scope lies in investigating the effect of other pre-analytical variables like storage temperature, centrifugal timing etc. on IL-6 assay.

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