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# SERUM PROTEINS OF GASTRIC CANCER PATIENTS

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**Abstract**

Serum proteins have a variety of uses, including immune system activity and lipid, vitamin, hormone, and mineral transfer in the circulatory system. Although serum proteins are present in very high quantities, their composition is unevenly distributed. This means that only 22 proteins, including serum globulins, albumin,and fibrinogen, make up 99% of all serum proteins, with the remaining 1% of blood proteins being made upof circulatory proteins with low abundance as well as proteins released by apoptotic, necrotic, and liver cells. Except for gamma globulins, which are made by the immune system, the liver, and bowels are responsible for secreting the majority of blood proteins. In the current research, we sought to assess the serum samples obtained from patients with gastric cancer and compare them to serum samples from healthy individuals. The serum samples were estimated using the Bradford method and performed SDS PAGE analysis. In light of protein-based studies using serum samples, this paper emphasizes thesignificance of serum sample collection, handling, and storage.
**Keywords:** Protein Estimation, SDS PAGE, Gastric Cancer, Protease inhibitors, Anticoagulants

## 1. INTRODUCTION

Human serum circulates in the blood as a carrier of exogenous and endogenous liquids. It enables things to bind to the serum's molecules and become embedded there. Thus, human serum aids in the transfer of thyroid hormones and fatty acids, which have an impact on the majority of body cells. Because they are readily available and stable, serum and plasma are useful resources for proteome

analysis, projects to find biomarkers, andresearch into the effects of human pathologies. [1,2]. Through blood, all bodily cells can interact. Numerous organelles and tissues within cells store nutrients and release some of their contents into the bloodstream, changing the blood's makeup. Because of this, this biological specimen is regarded as a crucial source of physiological data about the general health of each tissue and can provide information about the state of various pathological conditions.

One of the most intricate parts of the human proteome is the blood proteome. Its content represents each subject's physiological and pathological condition. The relatively easy sample removal is one of the most significant benefits of blood samples. Even though collecting urine or saliva is simpler, these samples have lower informational value, are less consistent, and vary more depending on the individual's behaviours and the time of day.

Salts, lipids, amino acids, carbohydrates, and a variety of proteins are all components of blood makeup. It includes more than 10,000 different protein classes and gathers proteins from tissues and organs throughout the entire body [3]. However, these figures only partly capture the true complexity of protein species, which is determined by the enormous diversity of proteoforms and antibody classes. About 90% of the proteins in serum and plasma are in large concentrations (HAPs). At least 50% of the overall protein content is made up of albumin, and the remaining 40% of the plasma proteome is primarily made up of fibrinogen and haptoglobin [4]. The presence of these larger proteins' degraded forms, proteins generated from genetic polymorphisms, and a significant amount of posttranslational modifications (PTMs) all contribute to the complexity of the serum/plasma proteome [5]. HAPs are frequently disregarded as potential biomarkers [6],but their relative abundance and cleaved or modified forms may accurately reflect the physiological and pathological state.

## MATERIALS AND METHODS

### Sample collection

All the study subjects included in this study were of South Indian Tamil origin and the ethical approval was granted by the Institutional Ethical Committee of Madras Medical College (MMC), Chennai (EC Reg No. ECR/270/Inst./TN/2013; IEC No. 31082015). Informed written permission was obtained from patients with primary GC (n=6) who were admitted to the Department of Medical Gastroenterology at MMC Chennai. A self- described fit person (n = 6) who is unaffected by gastrointestinal conditions or other cancers was chosen as the study's control. Centrifuging at 3500 rpm for 8 minutes isolated the serum from the whole blood sample. The liquid was later gathered and kept at -80ºC pending further processing.

## RESULTS AND DISCUSSION

### Sample Collection

### Modes of sample withdrawal

Sample collection mode is a crucial stage for two aspects, one of which is connected to patient withdrawal and the other to laboratory practice. The application of the tourniquet, the location of withdrawal, the patient's posture (whether he is upright, lying, or seated), and the use of alcohol to clean the skin can all result in hemolysis and have an impact on the proteomics analysis [7, 8]. Blood was drawn in the morning, just before 10 a.m., while the patient was fasting to prevent the diurnal fluctuations of blood biomarkers [9]. To reduce the risk of hemolysis, a 21-G needle is recommended during the draw step. The favored vein is the median cubital vein because it is typically simple to locate and access.

### Anticoagulants

Although managing plasma without clotting takes time, additional safety measures are required. Choosing serum or plasma for a biomarker study, and if plasma, deciding whichanticoagulants to use, is a crucial step in ensuring correct sample preparation. By the action of the proteases of thrombin on fibrinogen and on the other elements of the coagulation cascade, plasma can be spontaneously converted to serum at room temperature (RT) [10, 11 & 12]. To prevent plasma samples from clotting,

anticoagulant use (EDTA, heparin, citrate) is required during plasma separation.

HUPO (Human Proteome Organization) emphasized the need for uniform standards for each anticoagulant [13]. The preferred anticoagulant for a quantitative proteomics study appears to be EDTA [14]. However, generally, the single type of experiment needs to be evaluated because the use of anticoagulants may impact the stability of some particular proteins [15]. In reality, the anticoagulant may have some negative side effects [16]. For instance, the heparinized samples appear to be more stable [11]; as a result, heparin can interact with a wide range of various proteins in addition to the antithrombin III factor [17].Additionally, because it is a highly charged molecule, it can affect how other molecules in the solution bind to it, making it crucial in some specific methods like chromatographic separation [11, 15]. A popular anticoagulant with a negative charge is EDTA. It is not advised to use EDTA in the studies due to the use of divalent cations because it can bindto metal ions decreasing their reactivity. Although EDTA appears to be less stable than heparin [18], it provides the best method for inhibiting proteases that require metal ions for the coagulation process [14]. Another drawback is related to EDTA's capacity to causeplatelet clumping, which modifies the plasma proteome composition [19]. The other prevalent anticoagulant, citrate, which can bind calcium and is frequently present in fluid form in the tubes, causes a dilution effect when blood is added during sample preparation[13]. To avoid over-diluting the sample, it is crucial to determine the proper anticoagulant/blood ratio in this situation.

**Protease inhibitors**

Protein inhibitors should be introduced to the samples to stop protein degradation. Protease inhibitor use should begin during the period of sample collection, according to HUPO HPP. It has been shown through analysis of various peptide peaks acquired by SELDI-TOF-MS that plasma proteomics profiling of samples treated with inhibitors is more stable compared to the other untreated samples [13]. Plasma heparin and plasma EDTA were found to be the most proteolytically active substances, followed by serum and plasma citrate. However, it has been shown that adding protease inhibitors to samples ofheparinized EDTA and citrated results in a change in the pI profile evaluable. The activesites of proteases like trypsin and chymotrypsin can be modified by these compounds. Additionally, they can introduce an amine group to serine residues on other proteins, changing them and raising the pI. By spreading 2D gel, all of these changes can be assessed [10].

**Collection tubes**

The proteome composition, however, may change depending on the circumstances surrounding the sample collection. Silicones, plastic covering, polymeric surfactants (polyethylene glycols or polyvinylpyrrolidone), and polymeric gels can adjust viscosity, release from tubes, and change the serum/plasma composition, interfering with sample peaks that can be detected using MALDI spectra [20]. Additionally, it has been shown that the same sample can display a different proteomics profiling when it is collected in various tubes, such as red top tubes or tiger typo tubes [21, 22]. It has been discovered that collection tubes pre-loaded with a protease inhibitor cocktail and anticoagulants yield repeatable plasma samples [23]. In contrast, there are currently no commercially available serum collection tubes that can reliably generate serum samples for proteomicsresearch. Due to this, HUPO has advised using plasma rather than blood, and other massspectrometry groups concur [24, 25].

**Sample Stability and storage**

The plasma proteome is stable to sample preparation delays of up to one week at 4 °C, 25 rounds of freezing and thawing, and hemolysis [26]. Current and pertinent multicentric research conducted by Mateos et al., [27] supports these findings. Although there is a lotof stability, research by [27] and [28] shows that the concentrations of some proteins, such as plasminogen, transthyretin, and apolipoprotein, which are involved in some pathways with a circadian response, vary somewhat throughout the day. It would be preferable to gather the sample on the day of the test itself [29].

Differences in serum proteome are directly related to storage temperature and clotting time [21]. Peptides may be destroyed and novel peptides created during the coagulationprocess. According to [30],

serum peptide analysis could change when blood starts to clot(30–60 min). When the sample was kept at room temperature (RT) rather than on the ice, the blood proteomics profiling was only found to have changed after 60 min. According toHUPO's guidelines, serum should be obtained 60 min after clotting at room temperatureand then stored at 4-8°C, ideally at -80 °C, before analysis.

**Temperature**

The temperature is the key factor for the stability of the proteome and enzymatic activity, in the entire analysis, from the withdrawal to the collection and the transport to the storage, as highlighted also for other fluids [31, 32 & 33]. The importance of it for collection, transportation procedures, and freeze-thaw cycles is clear from the impacts on sample quality. These crucial elements have been the subject of numerous investigations[34].

Rai et al., [13] claim that liquid nitrogen storage is optimal for ensuring protein stability. The temperature should be set at 80°C directly after handling, preferably in small aliquots,if this is not possible [35]. Dry ice and ice packs should be used for transportation. Avoidlimiting storing cycles to just two refreezing phases.

**Sample Selection**

The idea that serum and plasma are the same liquid is untrue. From the perspective of clinical chemistry, serum, and plasma are only different from one another in that the latter contains fibrinogen while the former primarily varies in protein concentration and makeup. Plasma and serum protein and peptide levels have been found to differ [36 &18].

**SERUM SAMPLE PREPARATION & SDS PAGE ANALYSIS**

**Protein estimation**

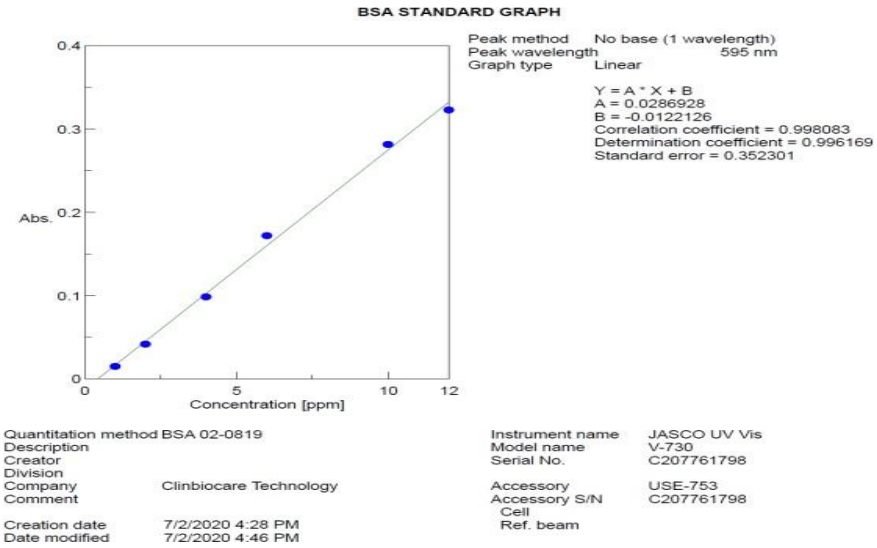
The standard graph must be created to estimate the protein concentration of serum samples. We created a standard graph using bovine serum albumin (BSA) as a reference protein, which will help us determine the concentration of serum samples. The standards'known concentrations were created using working stock BSA at a value of 1 mg/ml. A total of seven standards were created, with concentrations ranging from 10 µg, 20 µg, 40µg, 80µg, and 160 µg. Following the preparation of the BSA standard, the appropriatevolume for each standard was added individually into the tubes and made up to 1000 µl using milli q water as shown in table 1.

Following the preparation of the standards, 1000 µl of Bradford reagent (Sigma Aldrich, Germany) was applied to the standards before being incubated for 10 minutes at room temperature in the dark. The samples were examined in a Jasco (Maryland, USA) UV - Dual Beam Visible Spectrophotometer Model V - 730 at 595 nm following the incubation time. The readings were taken using the analytical software Spectra Manager II, and figure 1 shows the standard graph that was produced.

**Table 1: Details for preparing the standards**

| Std Number | Volume of BSA working concentration (1 mg/ml) | The volume of Milli Q water | A known concentration of BSA in µg | Absorbance OD at 595 nm |
|---|---|---|---|---|
| STD 1 | 1 µl | 999 µl | 1 µg | 0.015 |
| STD 2 | 2 µl | 998 µl | 2 µg | 0.0416 |
| STD 3 | 4 µl | 996 µl | 4 µg | 0.0984 |
| STD 4 | 6 µl | 994 µl | 6 µg | 0.1719 |
| STD 5 | 8 µl | 992 µl | 8 µg | 0.2577 |
| STD 6 | 10 µl | 990 µl | 10 µg | 0.2813 |
| STD 7 | 12 µl | 988 µl | 12 µg | 0.3227 |
| BLANK | - | 1000 µl | - | 0.000 |

**Figure 2: Standard graph with BSA for protein estimation**




By applying this standard graph, the unknown serum samples were estimated and the concentration of the protein was detailed in Table 2.

**Table 2: Protein estimation for the serum samples**

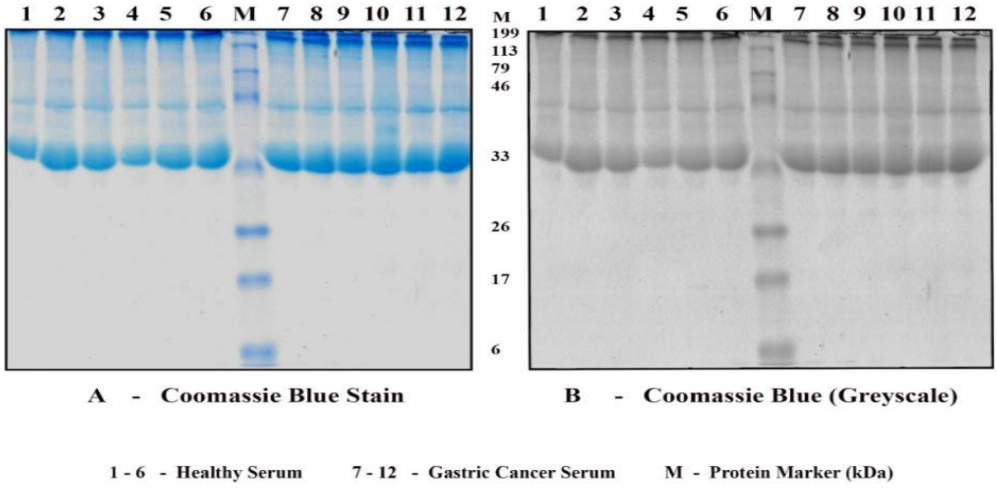
| Sample ID | Absorbance OD at 595 nm | Concentration of Protein in µg/µl |
|---|---|---|
| 1 | 1.52 | 52.75 |
| 2 | 1.53 | 52.94 |
| 3 | 1.50 | 51.88 |
| 4 | 1.43 | 49.48 |
| 5 | 1.52 | 52.52 |
| 6 | 1.65 | 57.00 |
| 7 | 1.54 | 53.11 |
| 8 | 1.62 | 55.87 |
| 9 | 1.59 | 54.83 |
| 10 | 1.49 | 51.39 |
| 11 | 1.56 | 53.80 |

| | | |
|---|---|---|
| 12 | 1.63 | 56.21 |

**SDS PAGE Analysis**

SDS PAGE analysis was used to further examine the estimated proteins and determine their quality and amount in the serum sample. Figure 2 displays the protein composition of serum samples taken from both healthy individuals and people with gastric cancer. Six samples of healthy people are represented by lanes 1 through 6, while six samples of gastric cancer cases are represented by lanes 7 through 12. The protein marker is added to learn about the molecular weight-dependent patterns of protein expression. To see the protein bands, the gels were stained with CBB 250 reagent.

**Figure 3: SDS PAGE analysis for serum samples**




**CONCLUSION**

A total of 22 proteins, including serum globulins, albumin, and fibrinogen, make up 99% of all serum proteins, with the remaining 1% of blood proteins being made up of circulatory proteins with low abundance as well as proteins released by apoptotic, necrotic, and liver cells. Bowels are responsible for secreting the majority of blood proteins. In the current research, we sought to assess the serum samples obtained from patients with gastric cancer and compare them to serum samples from healthy individuals.

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