Modification in Electrophoresis Instrument and Analysis of Its Efficacy

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Abstract
Electrophoresis is a key technique in biochemistry and molecular biology used to separate macromolecules by size and charge. This study aims to improve an electrophoresis instrument by modifying its design to boost functionality and efficiency. The key change involved reducing the size of the gel slab, which led to cost savings and improved separation resolution and reliability.
We evaluated the performance of the upgraded electrophoresis instrument by comparing it with a standard model using various DNA and protein samples. The results showed significant improvements in resolution, with clearer banding patterns and shorter run times. Statistical analysis confirmed that the enhanced instrument consistently delivered reliable and reproducible results across multiple trials.
Additionally, the modified instrument proved more versatile, accommodating a broader range of sample sizes and types. This enhancement extends its usefulness in both research and clinical diagnostics. Future work will focus on further optimizing the system's parameters and conducting validation studies with a variety of sample matrices to fully realize the potential of the upgraded electrophoresis instrument.

Key words: Electrophoresis, biochemistry, gel slab, modification

Introduction:
An electrophoresis instrument is an essential tool in molecular biology and biochemistry for separating and analyzing bimolecules based on their size, charge, or other physical properties in an electric field [1]. This overview provides a detailed look at the components, types, and applications of electrophoresis instruments. [2, 3]:

- **Components of Electrophoresis Instruments:**
- **Power Supply:** Delivers the electrical current and voltage required to create the electric field across the gel or capillary.
- **Electrophoresis Chamber:** Contains the gel or capillary where bimolecules separation takes place, and includes buffer reservoirs to maintain pH and conductivity.
- **Cellulose Paper Electrophoresis:** This method separates charged molecules, such as proteins or nucleic acids, by applying an electric field to a cellulose paper strip. Molecules migrate through the paper based on their charge and size and are subsequently visualized using specific stains.

- **Gel or Capillary Medium:**
- **Gel Electrophoresis:**
  - Agarose Gel: Used for separating large DNA fragments and RNA.
  - Polyacrylamide Gel (PAGE): Offers higher resolution for smaller DNA fragments and proteins.
- **Capillary Electrophoresis (CE):** Utilizes narrow-bore capillaries to achieve high-resolution separation of bimolecules based on charge and size.
- **Electrodes:** Anode and cathode electrodes create the electric field within the chamber or capillary. Made from materials like platinum, graphite, or stainless steel depending on the application.
- **Detection Systems:** UV/Vis absorbance detectors or fluorescent detectors are common for visualizing separated molecules. Imaging systems capture gel or capillary images for analysis.
- **Temperature Control:** Some instruments include temperature-controlled environments to stabilize the gel or capillary conditions, enhancing separation efficiency.

1. Types of Electrophoresis Instruments:

- **Cellulose Paper Electrophoresis:**

Cellulose paper electrophoresis utilizes the movement of charged particles through a cellulose fiber filter paper under an electric field. Separation occurs due to variations in the migration rates of molecules based on their charge and size. This technique is commonly employed for protein analysis, nucleic acid analysis, and enzyme analysis [4].

- **Gel Electrophoresis:**
- **Agarose Gel Electrophoresis:** Widely used for analyzing DNA and RNA [5].
- **Polyacrylamide Gel Electrophoresis (PAGE):** Ideal for analyzing proteins and smaller nucleic acids [6].Capillary Electrophoresis (CE):
  - Offers high separation efficiency and speed compared to gel-based methods [7].
  - Used in DNA sequencing, protein analysis, and pharmaceutical quality control.

Applications of Electrophoresis Instruments:
- **DNA Analysis:** Includes fragment sizing, genotyping, and DNA sequencing [8].
- **Protein Analysis:** Involves determining protein size, purity, and post-translational modifications [9].
- **Clinical Diagnostics:** Used for detecting genetic mutations, protein biomarkers, and disease-related biomolecules [10].
- **Forensic Science:** Applied in DNA profiling and identification for criminal investigations [11].Advancements and Challenges:

Advancements:
- **Automation and Integration:** Incorporation of automation and integration with other analytical techniques, such as mass spectrometry [12].
- **Microfluidic Systems:** Advancement in microfluidic systems for miniaturization and high-throughput analysis [13].

Challenges:
- Ensuring Result Reproducibility: Achieving consistent and reliable results.
- Optimizing Conditions: Tailoring conditions for specific molecules and samples.
- Effective Handling of Small Volumes: Managing small sample volumes efficiently.

Electrophoresis instruments are advancing with technological improvements, allowing researchers and clinicians to achieve greater resolution, sensitivity, and efficiency in bimolecules analysis [14]. These tools are crucial for deepening our understanding of genetics, proteomics, and various diseases, thus propelling progress in both biomedical research and clinical diagnostics.

Electrophoresis continues to be a fundamental technique in molecular biology and biochemistry, essential for separating bimolecules based on their charge and size. The ongoing development of electrophoresis instruments aims to improve their efficiency, resolution, and reliability. This study centers on modifying an electrophoresis instrument to enhance its performance and effectiveness in biomolecular analysis [15].

Modifying electrophoresis instruments is essential to overcoming current limitations and meeting the growing demands for higher resolution and faster analysis. This study focuses on reducing the size of the gel slab as a key modification. These changes are expected to address issues such as variability in separation results and lengthy experimental run times.

The effectiveness of these modifications is rigorously assessed through detailed comparative analyses. Various DNA and protein samples are used to evaluate the modified instrument's performance, including its ability to produce sharper banding patterns, shorten run times, and improve overall separation efficiency. Statistical techniques are employed to confirm the consistency and reproducibility of the modified instrument across multiple trials.

By enhancing both the performance and versatility of electrophoresis instruments, this study aims to advance biomolecular research and clinical diagnostics. The results are anticipated to guide further improvements in electrophoresis technology, facilitating more precise and efficient biomolecular analysis for a range of scientific and medical applications.

Materials and Methods:

Instrument Dimensions with image of instrument:
The instrument is constructed from an acrylic sheet (Figure 1 and Figure 2), with dimensions of 14.80 centimeters in length and 10.17 centimeters in width. The gel slab has a width of 2.8 centimeters, and the upper boundary of the gel slab measures 0.91 centimeters, shown in Figure 3. Operation of this instrument requires two wires for the cathode and anode, and a power supply of 100 volts is needed.

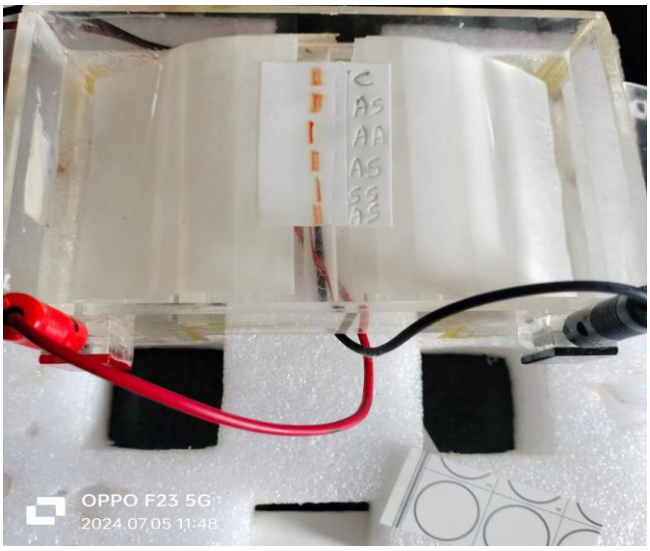

Figure 1: Top view of modified electrophoresis instrument with real-time test.

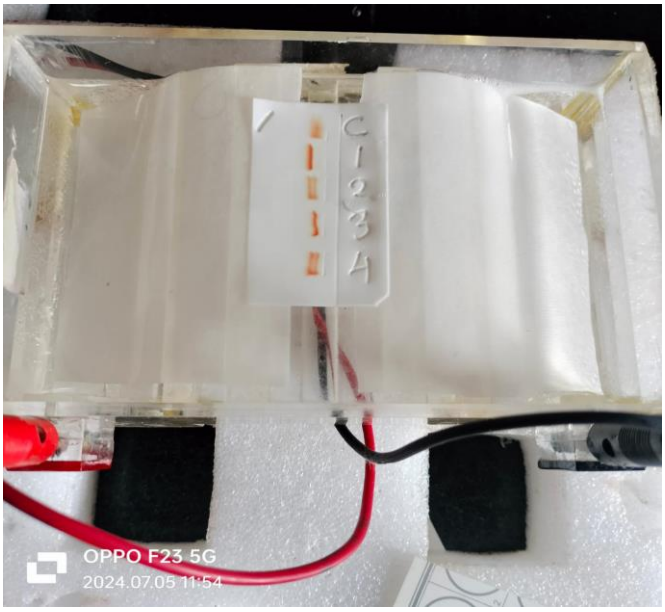

Figure 2: Real-time electrophoresis instrument SCD testing of 4 samples at a time

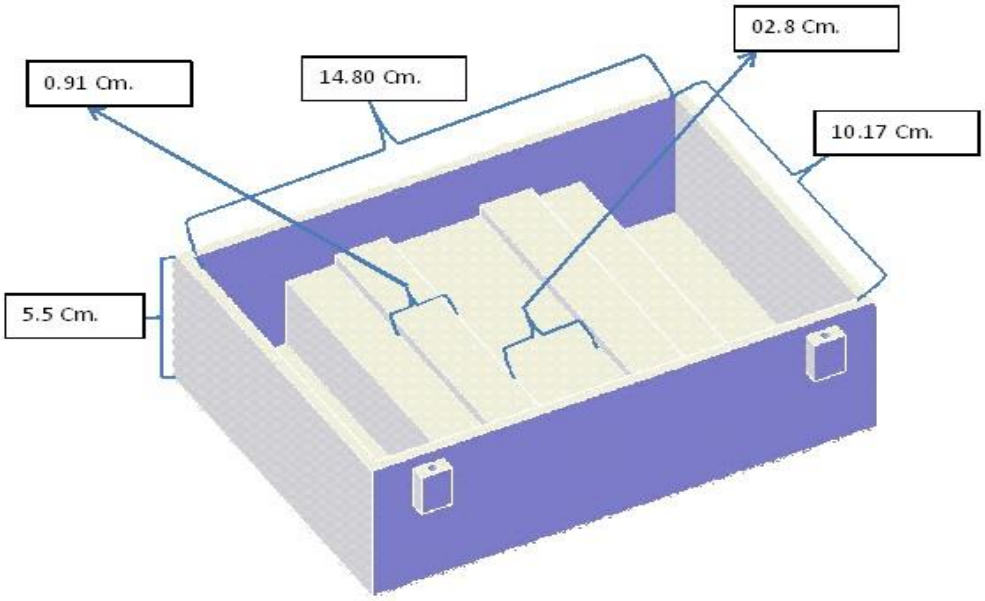

Figure 3: Diagram of modified Electrophoresis instrument with dimensions

Cellulose Acetate Electrophoresis at Alkaline pH:

Hemoglobin electrophoresis at pH 8.4-8.6 using a cellulose acetate membrane is a straightforward, reliable, and rapid method. It effectively detects most common clinically significant hemoglobin variants [16-18].

Principle: At alkaline pH, hemoglobin is negatively charged and will migrate toward the anode (+ve) during electrophoresis. Structural variants with changes in surface charge at alkaline pH will separate from Hb A. However, hemoglobin variants with amino acid substitutions located internally or those that do not alter the overall charge of the molecule may not separate effectively through electrophoresis.

Equipments: Electrophoresis Tank and Power Pack: Use any horizontal electrophoresis tank that accommodates a bridge gap of 7 cm. A direct current power supply that can deliver 350V at 50 mA is suitable for both cellulose acetate and citrate agar electrophoresis.

- **Wicks:** Use wicks made from filter or chromatography paper.
- **Blotting Paper:** Available from most electrophoresis equipment manufacturers, though fine micro capillaries can also be used [19].
- **Cellulose Acetate Membrane:** Plastic-backed membranes (7.6 x 6.0 cm) are recommended for their ease of use and storage.
- **Staining Equipment:** Use appropriate staining tools as required.

Reagents:

- **Electrophoresis Buffer:** Tris/EDTA/Borate (TEB), pH 8.5. Prepare by dissolving 10.2 g of Tris (tris (hydroxymethyl) aminomethane), 0.6 g of EDTA (disodium salt), and 3.2 g of boric acid in water to make a total volume of 1 liter. Store the buffer at 4°C; it remains usable for up to 10 runs without significant deterioration [20].
- **Wetting Agent:** For example, Zip-prep solution (Helena Laboratories). Prepare by adding 1 drop of Zip-prep to 100 ml of water.
- **Fixative/Stain Solution:** Ponceau S. Dissolve 5 g of Ponceau S and 7.5 g of trichloroacetic acid in water to make a total volume of 1 liter.
- **Destaining Solution:** Prepare a 3% (v/v) acetic acid solution by mixing 30 ml of acetic acid with water to make a total volume of 1 liter [21].
- **Hemolysing Reagents:** Use a 0.5% (v/v) Triton X-100 solution in 100 mg of potassium cyanide. Note that potassium cyanide is not included in the buffer preparation due to safety restrictions.

Method:

i. Centrifuge the sample at 1200 g for 5 minutes. Dilute 20 ml of packed red cells with 150 ml of hemolyzing reagent. Gently mix and let it sit for at least 5 minutes. For purified hemolysates, dilute 40 ml of a 10 g/dl hemolysate with 150 ml of lysing agent [22].
ii. With the power supply disconnected, prepare the electrophoresis tank by filling each outer buffer compartment with an equal amount of TEB buffer. Wet two chamber wicks with the buffer and place one along each divider/bridge support, ensuring good contact with the buffer.
iii. Soak the cellulose acetate by slowly lowering it into a buffer reservoir. Allow it to soak for at least 5 minutes before use.
iv. Fill the sample well plate with 5 ml of each diluted sample or control, and cover it with a 50 mm coverslip or a 'short' glass slide to prevent evaporation. Load the second sample well plate with Zip-prep solution.
v. Clean the applicator tips immediately before use by loading them with Zip-prep solution and then applying them to a blotter.
vi. Remove the cellulose acetate strip from the buffer and blot it twice between two layers of clean blotting paper. Ensure the cellulose acetate does not dry out.
vii. Load the applicator by dipping the tips into the sample wells twice and apply the first loading onto clean blotting paper. Reload the applicator and transfer the samples to the cellulose acetate.
viii. Position the cellulose acetate plates across the bridges with the plastic side facing up. Place two glass slides across the strip to ensure good contact. Electrophorese at 350 V for 25 minutes.
ix. After 25 minutes of electrophoresis, immediately transfer the cellulose acetate to Ponceau S staining solution and stain for 5 minutes.
x. Remove excess stain by washing in the first acetic acid reservoir for 5 minutes and then in each of the remaining two reservoirs for 10 minutes. Blot once with clean blotting paper and allow drying.
xi. Label the membranes and store them in a protective plastic envelope.

Interpretation and comments:

Figure 4 illustrates the relative electrophoretic mobilities of various hemoglobin variants at pH 8.5 on cellulose acetate. As shown in Figure 5, satisfactory separation of Hb C, S, F, A, and J is achieved. Typically, Hb S, D, and G migrate similarly, as do Hb C, E, and O^{Arab}. To differentiate these hemoglobins, techniques such as acid agarose gels, citrate agar electrophoresis, HPLC, or IEF can be used. Notably, there are subtle mobility differences between Hb S, Lepore, and D^{Punjab}, as well as between Hb C and E. Optimization of the technique can enhance the detection of these differences. Generally, Lepore Hb and Hb D^{Punjab} migrate slightly faster than Hb S, while Hb C migrates slightly slower than Hb E.

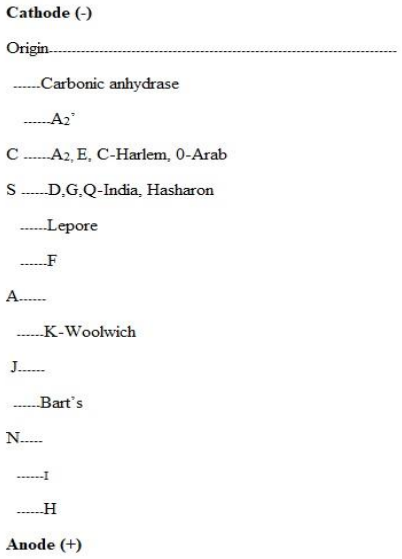

Figure 4: Schematic representation of relative mobilities of some abnormal heamoglobins. Cellulose acetate, pH 8.5

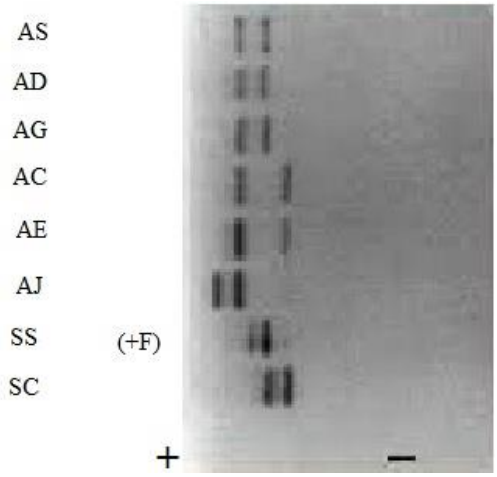

Figure 5: Relative mobilities of some abnormal heamoglobins. Cellulose acetate, pH 8.5

Any sample exhibiting a single band in either the S or C position should be further analyzed using acid agarose or citrate agar gel electrophoresis, HPLC, or IEF to rule out the possibility of compound heterozygotes such as SD, SG, CE, or CO^{Arab}. [23].

The quality of separation achieved with this procedure is mainly influenced by the quantity of hemoglobin applied and the placement of the origin. Delays between sample application and the start of electrophoresis, as well as issues such as improper staining after electrophoresis or inadequate blotting of the acetate prior to application, can lead to suboptimal results. This technique is sufficiently sensitive to differentiate Hb F from Hb A and detect variants of Hb A2.

If abnormal hemoglobin is detected, the presence of a Hb A2 variant band alongside the abnormal fraction indicates that the variant is an α-chain variant. Globin electrophoresis at both acid and alkaline pH can further clarify which globin chain is affected [24]. However, with the increasing availability of HPLC, this method is now less frequently required.

When abnormal hemoglobin is detected, measuring the percentage of the variant can be diagnostically important. This can be accomplished using electrophoresis with an elution procedure for Hb A2. Quantifying Hb S is particularly useful in clinical settings, such as for monitoring patients with sickle cell disease undergoing transfusion or diagnosing conditions where Hb S is co-inherited with α- and β-thalassemia, as detailed in Table1 Quantification of Hb S can be performed using HPLC, electrophoresis with elution, or micro column techniques. [25].

Table 1: Result of laboratories investigation in interactions of Hb S and a or b thalassaemia in adults

	MCV	**%S**	**%A**	**%A_2**	**%F**
AS	N	35-38	62-65	<3.5	<1
SS	N	88-93	0	<3.5	5-10
S/b^0 thalassaemia	L	88-93	0	<3.5	5-10
S/b^+ thalassaemia	L	50-93	3-30	<3.5	1-10
S/HPFH	N	65-80	0	<3.5	20-35

AS/a^{+} thalassaemia	N/L	28-35	62-70	<3.5	<1
AS/a^{0} thalassaemia	L	20-30	68-78	<3.5	<1
SS/a thalassaemia	N/L	88-93	0	<3.5	1-10
MCV mean cell volume; N, normal; L, low; HPFH, hereditary persistence of fetal haemoblobin.					

Results and Analysis:

The modification of electrophoresis instruments, involving the reduction in the size of the gel slab and cellulose acetate paper, is intended to enhance separation speed and overall efficiency.

- **Enhanced Resolution:** Smaller gel slabs can improve resolution when separating bimolecules like DNA fragments or proteins. Reduced migration distances minimize diffusion, resulting in clearer banding patterns and allowing for more precise differentiation between closely sized molecules.
- **Faster Run Times:** A smaller gel slab shortens the distance molecules must migrate, which reduces the duration of electrophoresis runs. This improvement can significantly increase laboratory throughput, enabling faster sample analysis.
- **Reduced Reagent Consumption:** Smaller gel slabs use less buffer and staining solutions, resulting in cost savings and less waste. This is especially beneficial in high-throughput environments where numerous samples are analyzed frequently.
- **Improved Heat Dissipation:** Smaller gel slabs typically offer better heat dissipation, helping to maintain stable electrophoresis conditions. This stability is essential for ensuring the reproducibility and reliability of experimental results.
- **Compatibility with Miniaturized Systems:** The miniaturization of electrophoresis instruments is becoming increasingly important for applications like point-of-care diagnostics and microfluidics. Smaller gel slabs enhance compatibility with these compact platforms, broadening the versatility and scope of electrophoresis technology.
- **Practical Considerations:** Sample Loading Optimization: Smaller gel slabs demand precise sample loading to optimize separation efficiency and achieve uniform banding patterns. Employing techniques such as well-loading guides or automated loading systems can improve accuracy and consistency.
- **Validation and Comparison:** To ensure the effectiveness of modifications, it's crucial to validate the performance of the updated instrument against standard methods. Comparative studies using established standards and reference samples can evaluate enhancements in resolution, run times, and reproducibility.
- **Application in Research and Diagnostics:** Modifying electrophoresis instruments by reducing gel slab size can significantly impact multiple scientific fields, including genetics, proteomics, and clinical diagnostics. These improvements advance molecular biology research and enhance diagnostic capabilities in healthcare settings.

After modifying the instrument, a total of 484 patients were tested using both the standard instrument and the modified one. Among them, 199 patients were SS and 284 were AS. The comparative results of these tests are presented in Figure 6. The analysis shows no difference in the results between the two instruments, indicating that the modified instrument maintains accuracy while improving efficiency.

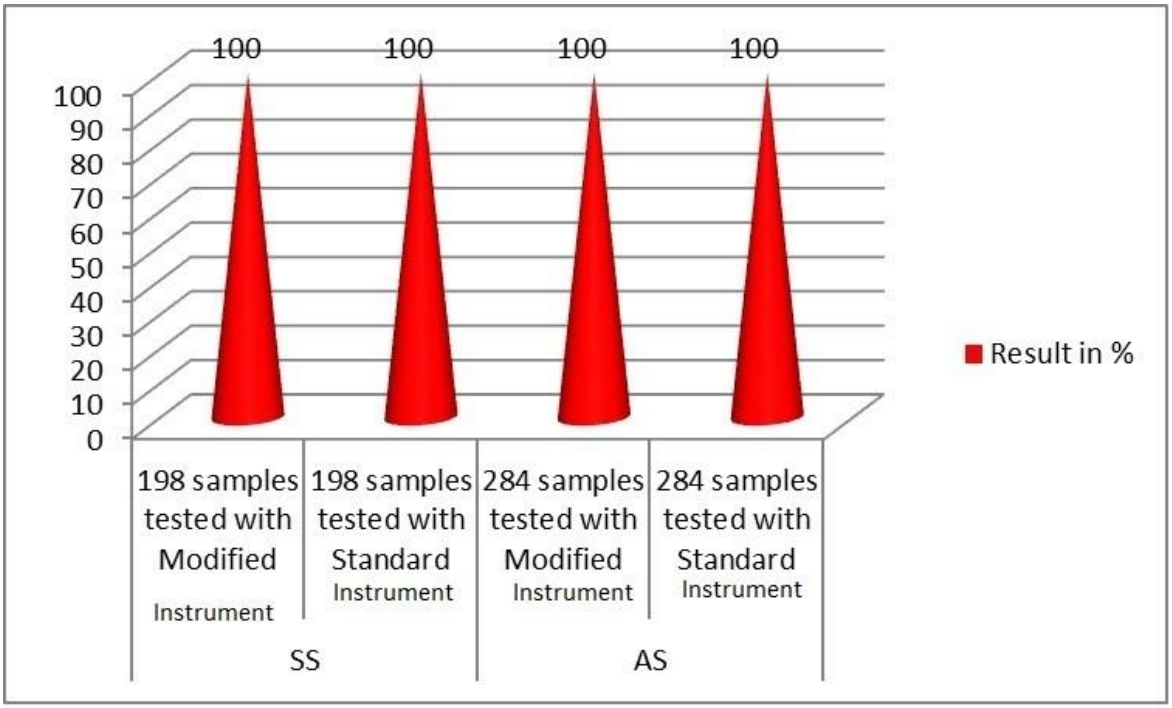

Figure 6 : Comparative Analysis of the samples tested with standard as well as modified instrumet

In summary, decreasing the size of the gel slab in electrophoresis instruments provides several advantages, such as enhanced resolution, quicker run times, lower reagent usage, and better heat dissipation. These enhancements facilitate progress in research techniques and diagnostic technologies, benefiting both scientific and healthcare fields.

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Availability of data and materials: This data has been generated as well as collected from hospitals by the scholar during research and it can be protected for study privacy. Due to ethical issues it cannot be disclosed.
Ethics declarations
We have taken the ethical approval from the Institutional Ethical Committee (IEC) (Ref. No. -125b/IEC/CIMS/BSP, dated: 03-12-2019).
Consent for publication
Not applicable.
Competing interests
The authors declare that they have no competing interests.

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