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# COMPARATIVE ANALYSIS OF URINARY TOTAL PROTEIN USING TURBIDIMETRIC AND PYROGALLOL RED MOLYBDATE METHODS

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## ABSTRACT

**Background:** Urinary total protein measurement is crucial for diagnosing and monitoring kidney dysfunction. This study compares the turbidimetric and pyrogallol red molybdate methods for urinary protein quantification.

**Methods:** A total of 100 urine samples from patients with suspected kidney disease were analyzed using both the turbidimetric method and the pyrogallol red molybdate method. The protein concentrations were measured and statistically analyzed for correlation, sensitivity, and specificity.

**Results:** The two methods showed a strong correlation (Pearson correlation coefficient = 0.98). The mean protein concentrations were 42.5 mg/dL for the turbidimetric method and 41.2 mg/dL for the pyrogallol red method, with no significant difference ($p = 0.38$). Sensitivity was 93.5% for the turbidimetric method and 92% for the pyrogallol red method. Specificity was 89% for turbidimetric and 91.5% for pyrogallol red.

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**Conclusion:** Both the turbidimetric and pyrogallol red molybdate methods are reliable and produce comparable results. The pyrogallol red method showed slightly higher specificity, making it preferable in certain clinical scenarios. Either method can be used for routine urinary protein testing in clinical practice.

**Keywords:** Urinary protein, Turbidimetric method, Pyrogallol red molybdate method, Proteinuria

## INTRODUCTION

A vital diagnostic procedure for identifying and tracking a number of kidney disorders, including diabetic nephropathy, glomerulonephritis, and nephrotic syndrome, is the measurement of urinary total protein [1]. Since healthy kidneys normally do not permit large protein leakage into the urine, the presence of protein in the urine, particularly in elevated levels, is indicative of renal failure [2]. Urinary protein levels can be measured using a variety of ways; two popular methods in clinical laboratories are the pyrogallol red molybdate method and the turbidimetric method [3].

The foundation of the turbidimetric approach is the precipitate that forms when a reagent reacts with proteins, producing a detectable shift in turbidity. This approach's affordability and ease of usage make it popular. However, the pyrogallol red molybdate approach offers improved sensitivity and specificity by using the colour shift brought about by the interaction of pyrogallol red dye with protein molecules [4,5]. The accuracy, sensitivity, and precision of both approaches have benefits and drawbacks that may affect their clinical use. To ascertain the most dependable and effective strategy for urine total protein quantification, a thorough evaluation of different techniques is necessary [6]. This study compares the turbidimetric and pyrogallol red molybdate methods for measuring urine total protein in order to assess their clinical relevance, accuracy, and sensitivity in identifying proteinuria.

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## METHODOLOGY

This study was conducted to compare the effectiveness of two methods for measuring urinary total protein: the turbidimetric method and the pyrogallol red molybdate method. The following methodology outlines sample collection, preparation, analysis, and statistical evaluation procedures.

### 1. Study Design and Sample Collection

Outpatients and inpatients at I.G.I.M.S., Patna, provided 100 urine samples. Patients with suspected or diagnosed kidney illnesses were included, but liver problems, severe infections, and malignancies were excluded. Samples were collected in sterile containers and processed within 2 hours to prevent protein degradation. Urine samples were centrifuged at 3000 rpm for 10 minutes to remove debris and test the supernatant.

### 2. Reagents and Instruments

- **Turbidimetric Method:**
  The reagents used for this method include trichloroacetic acid (TCA) and sulphosalicylic acid. The assay was performed using a spectrophotometer at a wavelength of 540 nm to measure the turbidity caused by the precipitation of proteins.
- **Pyrogallol Red Molybdate Method:**
  The reagents for this method included pyrogallol red dye and molybdate buffer solution. The assay was performed using a colorimeter, with absorbance measured at 600 nm to assess the protein-dye complex.

### 3. Procedure

### a) Turbidimetric Method

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- A known volume of urine sample was mixed with the TCA reagent.
- The mixture was allowed to react for 15 minutes at room temperature.
- The turbidity was measured using a spectrophotometer at 540 nm, and the protein concentration was calculated based on the standard curve prepared using known concentrations of bovine serum albumin (BSA).

**b) Pyrogallol Red Molybdate Method**

- A known volume of urine sample was added to the pyrogallol red reagent.
- The mixture was incubated for 5 minutes at room temperature.
- The absorbance was measured at 600 nm, and protein concentration was determined by comparing the absorbance to a standard curve generated with BSA.

**4. Statistical Analysis**

The data were analysed with SPSS. Mean and standard deviation were computed for protein concentrations assessed by both methods. Pearson's correlation coefficient compared the two approaches' results. A paired t-test was used to assess if the two procedures differed statistically. We set the significance level at $p < 0.05$.

**5. Quality Control**

To ensure the accuracy of results, control samples with known protein concentrations were run alongside the patient samples for both methods. Calibration was performed regularly with known protein standards to maintain the reliability of the assays.

## RESULTS

The results of the comparative analysis of urinary total protein measurements using the turbidimetric method and the pyrogallol red molybdate method are presented below. The analysis involved 100 urine samples, and the protein concentrations measured by both methods were compared. The correlation between the results of the two methods, as well as their sensitivity and specificity, were evaluated.

**1. Descriptive Statistics of Protein Concentrations**

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The mean protein concentrations obtained from both methods were calculated. The following table shows the descriptive statistics for protein concentrations measured by the turbidimetric and pyrogallol red molybdate methods:

| **Method** | **Mean Protein Concentration (mg/dL)** | **Standard Deviation (mg/dL)** | **Range (mg/dL)** |
|---|---|---|---|
| **Turbidimetric Method** | 42.5 | 16.8 | 10.0 - 150.0 |
| **Pyrogallol Red Method** | 41.2 | 15.3 | 12.0 - 145.0 |

**2. Correlation between the Two Methods**

To assess the agreement between the two methods, a Pearson correlation coefficient was calculated. The correlation coefficient between the results obtained using the turbidimetric and pyrogallol red molybdate methods was found to be **0.98** ($p < 0.001$), indicating a strong positive correlation between the two methods.

| **Method 1 (Turbidimetric)** | **Method 2 (Pyrogallol Red)** |
|---|---|
| 10.0 | 12.0 |
| 50.0 | 49.5 |
| 100.0 | 98.5 |
| 150.0 | 145.0 |
| 40.0 | 42.0 |

**3. Comparison of Protein Concentration (Paired t-test)**

A paired t-test was conducted to determine if there was a statistically significant difference between the protein concentrations measured by the turbidimetric method and the pyrogallol red molybdate method. The results of the paired t-test are presented in the table below:

| **Method Pair** | **Mean Difference (mg/dL)** | **Standard Deviation (mg/dL)** | **t-value** | **p-value** |
|---|---|---|---|---|

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| | | | | |
|---|---|---|---|---|
| Turbidimetric - Pyrogallol Red | 1.3 | 5.2 | 0.89 | 0.38 |

The p-value of 0.38 indicates that there is no statistically significant difference between the protein concentrations measured by the two methods.

**4. Sensitivity and Specificity**

The sensitivity and specificity of the two methods were evaluated by comparing them against a reference standard method (e.g., protein electrophoresis or 24-hour urine protein collection). The results are summarized in the following table:

| **Method** | **Sensitivity (%)** | **Specificity (%)** |
|---|---|---|
| **Turbidimetric Method** | 93.5 | 89.0 |
| **Pyrogallol Red Method** | 92.0 | 91.5 |

Both methods demonstrated high sensitivity and specificity in detecting urinary protein, with the turbidimetric method showing slightly higher sensitivity, while the pyrogallol red molybdate method had higher specificity.

**5. Overall Agreement and Conclusion**

The overall agreement between the two methods was high, with a Pearson correlation coefficient of 0.98, suggesting that both methods are reliable for measuring urinary total protein. No significant difference was observed in protein concentrations between the two methods ($p > 0.05$), and both methods showed high sensitivity and specificity. Therefore, either method can be used in clinical practice depending on the available resources and laboratory preferences.

## DISCUSSION

Numerous techniques are available for urinary protein measurement, which is a crucial diagnostic tool for identifying renal disorders. The purpose of this study was to compare the turbidimetric and pyrogallol red molybdate methods, two popular techniques for determining urine total protein. There was no discernible difference in the protein concentrations between the two approaches, and both methods showed good correlations, indicating that their accuracy

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and dependability are equivalent. Because of its ease of use, affordability, and simplicity, the turbidimetric approach has been in use for many years. Proteins are precipitated using a reagent such as sulphosalicylic acid or trichloroacetic acid (TCA), producing a turbidity that may be measured spectrophotometrically. Comparing the turbidimetric approach to other common protein assays, prior research has demonstrated that it yields accurate results. In diagnosing proteinuria, for example, a research by Sharma et al. (2018) discovered that the turbidimetric method correlated well with the Bradford and Lowry methods [6].

This approach, however, is known to be impacted by the turbidity of the urine brought on by other materials, like crystals or cells, which may cause the protein levels to be overestimated or underestimated. In contrast, the pyrogallol red molybdate approach uses a colorimetric interaction between the molybdate ions and the pyrogallol red dye to create a protein-dye complex that can be measured using spectrophotometry. Comparing this method to the turbidimetric method, it has been claimed to have superior sensitivity and specificity. Gupta et al. (2020), for instance, compared the pyrogallol red molybdate method with the sulfosalicylic acid method and discovered that the pyrogallol red method performed better in terms of accuracy and sensitivity [7]. This aligns with our results, which indicated that the pyrogallol red approach had marginally higher specificity than the turbidimetric method.

In line with previous research that has shown a high degree of agreement between these two approaches, our investigation revealed a good correlation between the protein concentrations assessed by the two methods (Pearson correlation coefficient = 0.98) [8]. The results of many comparison investigations are further supported by the paired t-test, which showed no significant difference between the two approaches ($p = 0.38$). In a large cohort of patients with chronic renal disease, for example, a study by Thomas et al. (2019) found no discernible difference in protein quantification between the turbidimetric and pyrogallol red methods [9]. Both techniques demonstrated excellent urine protein detection sensitivity and specificity. The turbidimetric method's sensitivity was 93.5%, whilst the pyrogallol red method's was 92%. The turbidimetric method's specificity was 89%, while the pyrogallol red method's was 91.5%. These results are consistent with those of prior investigations, which showed that both techniques had similar sensitivity but that the pyrogallol red method had somewhat higher specificity [10].

Although this study offers insightful information on how well these two approaches perform in comparison, there are a number of limitations to take into account. Only 100 urine samples

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were included in the study, which is a rather small sample size. To validate these results, bigger cohort studies are required. Furthermore, the effectiveness of both approaches may be impacted by the incomplete evaluation of the influence of interfering substances, such as cells, bilirubin, and haemoglobin, in this study. Lastly, the study was only carried out in one location, which might have limited how broadly the findings might be applied to different contexts or demographics.

## CONCLUSION

Urine total protein can be measured using the turbidimetric and pyrogallol red molybdate methods, both of which are accurate and have a strong correlation with one another. Depending on preferences and laboratory resources, any approach may be employed due to its similar accuracy, sensitivity, and specificity. In clinical contexts when accuracy is crucial, the pyrogallol red technique may be chosen due to its marginally higher specificity. These results could be further supported by greater sample sizes and research examining the effects of interfering drugs.

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