Effects of different preservation methods on pH, glucose and protein in urine

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Abstract - Delay in transport of urine specimens from collection site to analytical laboratories causes significant errors. Preservatives conserve the characteristics of the urine. The objective of the study was to compare the effect of different preservations on pH, glucose and protein in urine. Collected urine samples from normal individuals were aliquoted for different preservation methods after adding glucose (20, 40, 60, 80, 100 mg/dL) and albumin (20, 30, 100, 300, 500 mg/dL) and stored for 0, 6, 24, 48 and 72 hours. The pH, glucose and protein were measured using pH meter, glucose oxidase, sulfosalicyclic acid methods respectively. Urine samples stored without preservatives showed statistically significant differences ($p<0.05$) for pH, glucose and protein at 6 hours with 0 hour sample. The pH of urine samples stored at 4°C showed significant differences at 72 hours ($p<0.05$). Added glucose (20 mg/dL) and added protein (20 mg/dL) of urine samples stored at 4°C showed significant difference at 24 hours ($p<0.05$). Urine samples with 40, 60, 80 and 100 mg/dL 'added' glucose and those with 30, 100, 300 and 500 mg/dL 'added' protein stored at 4°C showed significant differences at 6 hours ($p<0.05$). Urine samples stored at 25°C with thymol or toluene showed significant differences at 6 hours ($p<0.05$) for pH, glucose and protein. Urine samples without preservatives showed significant differences when compared with urine samples at 4°C, either with thymol or toluene ($p<0.05$). Urine samples stored at 4°C showed significant differences when compared with urine samples either with thymol or toluene ($p<0.05$). Urine samples with thymol showed no significant differences for pH ($p>0.05$) while showing significant differences in glucose and protein when compared with urine samples with toluene ($p<0.05$). The preservation of the urine samples at 4°C is superior to the chemical preservatives and without preservatives. If refrigeration is unavailable, then it is recommended to use either toluene or thymol. Among the two preservatives, toluene is better to preserve glucose and thymol is better to preserve protein. Thymol has strong antibacterial attributes whereas toluene acts as a physical barrier to air and bacteria.

Keywords: Glucose, pH, Protein, Urine preservatives

I. INTRODUCTION

Urinalysis is the non invasive, most helpful and cost effective diagnostic test and is commonly used to monitor and detect various human diseases. Kidneys play the major role in maintaining pH of the body [1]. Evaluation of urine pH is required to identify acid-base disturbances in human. In addition, blood glucose level and plasma protein level are affected in kidney diseases. As a result, the glucose and protein appear in urine. Normal concentration of glucose in urine is 0.1 to 0.8 mmol/L [2]. The presence of glucose above normal limit in urine is known as glucosuria. Normal urine contains up to 1 to 14 mg/dL of protein [1]. Presence of protein in urine above normal limit is known as proteinuria [2]. However, in normal healthy individuals, the above mentioned biochemicals could be altered due to delay in analysis. To eradicate such errors, the preservatives are used to conserve the characteristics of urine, prevent inaccurate results and to reduce bacterial action or chemical decomposition [2]. Toluene, thymol, chloroform, phenol, formalin and refrigeration at 2-8oC are commonly used [4]. The easiest means of preserving urine specimen is refrigeration. Refrigeration preserves the properties of urine by preventing bacterial decomposition and preserves cellularity for a prolonged time. Thymol has phenolic structure and posses antibacterial attributes. This antibacterial activity is caused by inhibiting growth and lactate production and decreasing cellular glucose uptake by bacteria. Toluene merely lies on the surface of the urine, forming a thin layer and acting as a physical barrier to air and bacteria. Several studies show that, the preservatives alter the quantities of biochemicals. The objective of this study was to compare the effect of different preservation methods on pH, 'added' glucose and 'added' protein in urine for 6, 24, 48 and 72 hours.

II. METHODOLOGY

Urine samples were collected from healthy volunteers. A total of 300 mL urine sample was collected from six healthy individuals who were available at the time of sample collection. Approximately 40-50 mL of urine samples were collected from each individual according to the standard procedures. The urine samples were pooled. To the urine samples either glucose or albumin was added to make the urine glucose concentrations to 20, 40, 60, 80 and 100 mg/dL and urine protein concentrations to 20, 30, 100, 300 and 500 mg/dL. Urine samples were divided into four. Each of this was preserved either without preservatives at 25°C or in refrigerator without chemical preservatives at 4°C or with thymol or with toluene. The urine samples added with chemical preservatives were stored at 25°C. The samples were analyzed for pH, glucose and protein at 0, 6, 24, 48 and 72 hours in triplicates. The pH was measured using pH meter. Glucose and protein were measured using glucose oxidase [5], sulfosalicyclic acid [3] methods respectively. Ethical clearance was obtained from the Ethics Review Committee of the Faculty of Medicine, University of Jaffna.

All data collected in this study were entered in Statistical Package of Social Science (SPSS) Version 21. The descriptive statistics (mean and standard deviation), mean comparison with one way ANOVA and Pearson correlation were used for analysis in this study.

III. RESULTS AND DISCUSSION

Without preservatives

Urine samples without preservatives have shown significant differences in pH, 'added' glucose concentrations (20, 40, 60, 80 and 100 mg/dL) and 'added' protein concentrations (20, 30, 100, 300 and 500 mg/dL) than that of 0 hour sample ($p<0.05$) at 6 hours.

Refrigeration at 4°C

Urine samples stored at 4°C did not have significant difference in pH up to 48 hours ($p>0.05$) while significantly changed at 72 hours than that of 0 hour samples ($p<0.05$). The changes in glucose concentrations (20 mg/dL) and protein concentrations (20 mg/dL) were statistically not significant at 6 hours ($p>0.05$) while significantly changed at 24 hours ($p<0.05$). There were statistically significant differences at 6 hours in 'added' glucose concentrations (40, 60, 80 and 100 mg/dL) and 'added' protein concentrations (30, 100, 300 and 500 mg/dL) in comparison with 0 hour samples ($p<0.05$).

Thymol as preservative

The changes in pH , different 'added' glucose concentra-tions and different 'added' protein concentrations were significant at 6 hours of storage in the urine samples preserved with thymol than that of 0 hour samples ($p<0.05$).

Toluene as preservative

The changes in pH, 'added' glucose concentrations and 'added' protein concentrations were significant at 6 hours of storage in the urine samples preserved with toluene than that of 0 hour samples ($p<0.05$).

Comparison between different preservation methods

The changes in pH were significant in samples stored without preservatives compared with the samples which were stored at 4°C ($p<0.05$). Urine samples either with 'added' glucose or 'added' protein stored at 4°C have shown significant differences when compared with the urine samples without preservatives ($p<0.05$). Urine samples stored without preservatives have shown significant differences in pH, 'added' glucose concentrations and 'added' protein concentrations when compared with urine samples stored with thymol ($p<0.05$). Urine samples stored with toluene have shown significant differences in pH, 'added' glucose concentrations and 'added' protein concentrations when compared with urine samples stored without preservatives ($p<0.05$). Urine samples stored at 4°C have shown significant differences in pH, 'added' glucose concentrations and 'added' protein concentrations when compared with urine samples stored with thymol ($p<0.05$). Urine samples stored at 4°C have shown significant differences in pH, 'added' glucose concentrations and 'added' protein concentrations when compared with urine samples stored with toluene ($p<0.05$). The changes in pH values were not significant in urine samples stored with thymol when compared with urine samples stored with toluene ($p>0.05$). Urine samples stored with thymol have shown significant differences in 'added' glucose concentrations and 'added' protein concentrations when compared with urine samples stored with toluene ($p<0.05$). The mean glucose concentrations of urine samples stored with toluene were higher than mean glucose concentrations of urine samples stored with thymol. The mean protein concentrations of urine samples stored with thymol were higher than mean protein concentrations of urine samples stored with toluene.

Discussion

Fig.1 and fig.2 indicate that the difference percentage for pH of urine increases with time. The difference percentages in urine pH were less in refrigeration, thymol, toluene, without preservatives respectively. It is likely due to bacterial action which metabolizes urea to ammonia.

Fig.3 and fig.4 reflect that the difference percentage for glucose in urine increases during storage. The difference percentage in glucose concentration was less in refrigeration, toluene, thymol and without preservatives respectively. It is due to the consumption by cells and bacteria present in urine.

Fig.5 and fig.6 indicate that the difference percentages for protein in urine increases during storage. The difference percentage in protein concentration was less in refrigeration, thymol, toluene and without preservatives respectively. It is due to digestion by the endogenous urinary proteases.

Refrigeration preserves the properties of urine by pre-venting bacterial decomposition and preserves cellularity and does not interfere with the chemical testing. Thymol has strong antibacterial attributes because of its phenolic structure. Toluene merely lies on the surface of the urine, forming a thin layer and acting as a physical barrier to air and bacteria. Based on our literature review, there was no other study conducted to compare urine pH, glucose and protein in samples preserved with chemical preservatives (thymol, toluene) versus refrigeration.

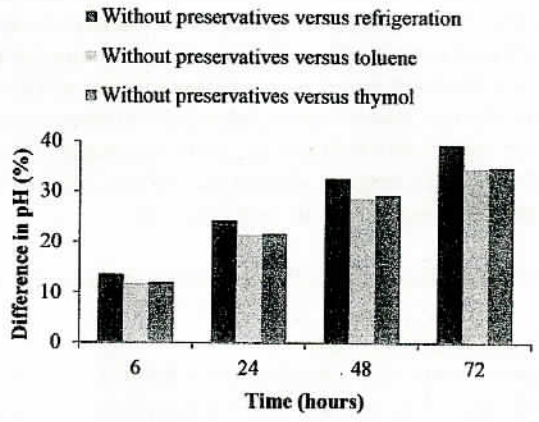


Fig.1: Difference between preservation methods for urine pH

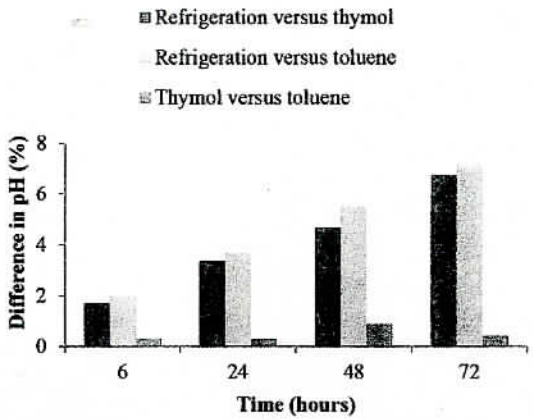
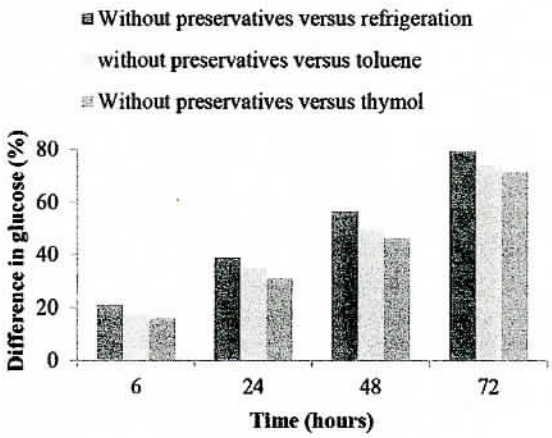
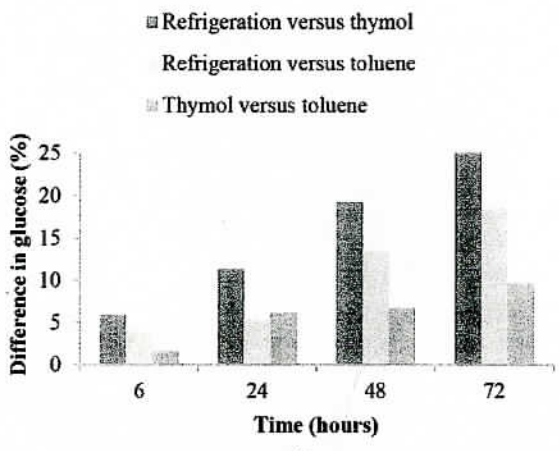


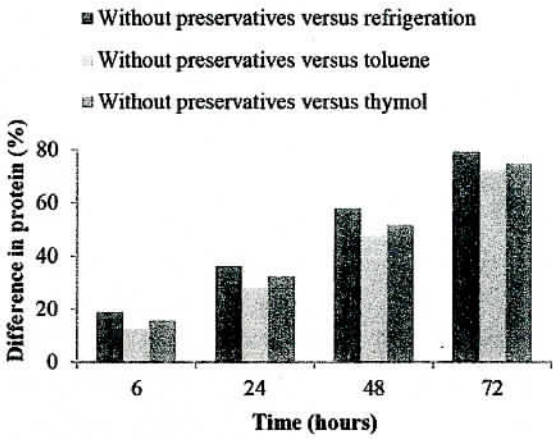
Fig.2: Difference between preservation methods for urine pH



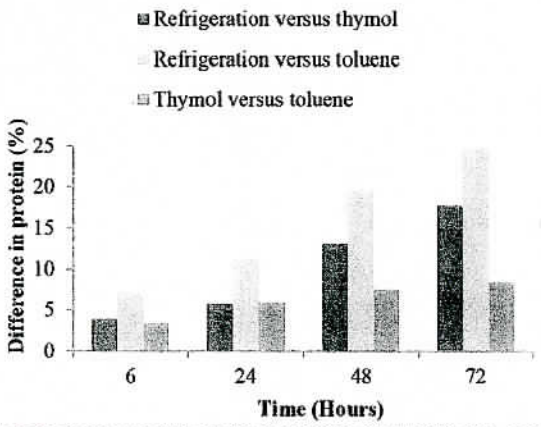
glu-cose of 20 mg/dL (same pattern follows for 40, 60, 80 and 100 mg/dL)



glu-cose of 20 mg/dL (same pattern follows for 40, 60, 80 and 100 mg/dL)



pro-tein of 20 mg/dL (same pattern follows for 30, 100, 300 and 500 mg/dL)



pro-tein of 20 mg/dL (same pattern follows for 30, 100, 300 and 500 mg/dL)

IV. CONCLUSION

Based on this study, it can be concluded that preservation of the urine samples by refrigeration for pH, glucose and protein analysis is superior to the addition of thymol or toluene and storing at 25°C or without preservatives. If refrigeration facility is unavailable, it is recommended to preserve the urine samples for pH with toluene or thymol. Urine samples for glucose analysis to be preserved with toluene over thymol and urine samples for protein analysis to be preserved with thymol over toluene for up to 72 hours when compared with storing the urine samples without preservatives.

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