

## Effect of Serum-Clot Contact Time on Some Biochemical Constituents of Sheep Blood

Mahmoud R. Abd Ellah, Nourhan S. Tawfeek, Gehan A. Ali, Enas M. Ibrahim, Wafaa H. Mohamed, Eman M. Sadiek, Maram A. Mahmoud, Doaa H. Allam, Dena A. Abd-Elzaher, Radwa H. Ahmed, Mona M. Mohamed, Mahetab M. Mohamed and Raafat W. Eskander

Department of Animal Medicine, Faculty of Veterinary Medicine, Assiut University, 71526 Assiut, Egypt

**Abstract:** To assess the changes that occurs in serum constituents that kept in contact with the clot with time and under different storage temperature. The clotted specimens were allowed to stand at 4 or 30°C for 0, 3, 6 or 24 h and then assayed. Three rams were used in the study, blood samples were collected via jugular vein and divided into 7 plain tubes (totally, 21 blood samples used). Three tubes were centrifuged within 30 min of dispensing the aliquots, these sera represented initial (zero time) values. The remaining 18 aliquots were divided into two equal groups; one group was stored at 4°C in a refrigerator and another one at 30°C in an incubator. Serum kept in contact with the clot at 30°C showed significant decreases in serum albumin level and significant increases in serum globulin levels at 24 h. Serum kept in contact with the clot at 4°C showed significant increases in creatinine levels at 3 and 6 h and significant increase in serum phosphorus level at 24 h. In conclusion, serum samples for total protein, BUN and magnesium can be harvested after 24 h storage at room temperature or refrigerator. For creatinine serum must be harvested before 3 h storage at 4°C, for albumin and globulins serum must be separated before 24 h at room temperature.

**Key words:** Serum-clot, rams, storage, temperature, measurement, Egypt

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### INTRODUCTION

Proper blood sample processing and handling is required in order to obtain reliable biochemical measurements. Temperature and duration of whole blood storage can affect some biochemical constituents. To avoid time-dependent changes in the release of cellular constituents, it is standard practice to separate serum from the blood clot by centrifugation as soon as the clot forms (Ono *et al.*, 1981; Stokol and Nydam, 2005).

In veterinary medicine, very little has been published on the stability in whole blood and of many biochemical markers. The issue is important because in large epidemiological studies, it is often not feasible to centrifuge and freeze blood samples immediately after vein puncture. To assess the changes that occurs in serum constituents with time and under different storage temperature. The clotted specimens were allowed to stand at 4 or 30°C for 0, 3, 6 or 24 h and then assayed.

### MATERIALS AND METHODS

Three rams that belong to the Veterinary Teaching Hospital, Assiut University were used in the study, blood

samples were collected via jugular vein and divided into 7 plain tubes (totally, 21 blood samples used). Three tubes were centrifuged within 30 min of dispensing the aliquots. These sera represented initial (zero time) values. The remaining 18 aliquots were divided into two equal groups; one group was stored at 4°C in a refrigerator and another one at 30°C in an incubator.

At 3, 6 and 24 h from initial zero time sampling, three aliquots from each temperature group were centrifuged and the separated sera were kept frozen at -20°C until assay. Serum analysis was performed within the same week of collection. The harvested sera were used for measuring total proteins (g dL<sup>-1</sup>), albumin (g dL<sup>-1</sup>), globulins (g dL<sup>-1</sup>), creatinine (mg dL<sup>-1</sup>), Blood Urea Nitrogen (BUN, mg dL<sup>-1</sup>), phosphorus (mg dL<sup>-1</sup>) and magnesium (mg dL<sup>-1</sup>) using commercially supplied kits (Spectrum-diagnostics, Cairo, Egypt) and by means of Digital VIS/Ultraviolet Spectrophotometer (Cecil instruments, Cambridge, England, Series No. 52.232).

**Statistical analysis:** Statistical analysis was conducted using SPSS 16.0 for windows (SPSS, Chicago, USA). Statistical analysis was performed by comparing data from 3, 6, 24 h with those from 0 h using one-way analysis of

variance (repeated measures ANOVA). Data at different storage temperature were compared using one way ANOVA. Statistically significant differences were determined at  $p \leq 0.05$ . Data were expressed as Mean $\pm$ SD.

## RESULTS AND DISCUSSION

**Effect of storage at room temperature:** There were significant decreases in serum albumin level and significant increases in serum globulin levels after 24 h. On the other hand, serum levels of total proteins, creatinine, BUN, phosphorus and magnesium showed insignificant changes at 3, 6 and 24 h when compared with 0 h group (Table 1).

**Effect of storage at refrigerator:** There were significant increases in creatinine levels at 3 and 6 h and significant increase in serum phosphorus level at 24 h. On the other hand, serum levels of total proteins, albumin, globulins, BUN and magnesium showed insignificant changes at 3, 6 and 24 h when compared with 0 h group (Table 2).

The results of the present study demonstrated that sheep serum kept in contact with the blood clot for as long as 24 h and at different storage temperatures had no significant effect on the measurements of serum total proteins and BUN levels. Serum albumin significantly decreased at 24 h after incubation with the blood clot at room temperature (Table 1). The non-significant increase in serum total proteins is in accordance with that reported by Ehsani *et al.* (2008) for cattle blood and disagreed with Ono *et al.* (1981) for human blood.

Table 1: Effect of clot contact time on serum biochemical parameters stored at 30°C

| Parameters                          | Hours (No = 3)   |                  |                  |                   |
|-------------------------------------|------------------|------------------|------------------|-------------------|
|                                     | 0                | 3                | 6                | 24                |
| Total protein (g dL <sup>-1</sup> ) | 6.48 $\pm$ 0.44  | 6.78 $\pm$ 0.48  | 6.66 $\pm$ 0.48  | 6.39 $\pm$ 0.29   |
| Albumin (g dL <sup>-1</sup> )       | 2.62 $\pm$ 0.06  | 2.65 $\pm$ 0.07  | 2.90 $\pm$ 0.22  | 1.67 $\pm$ 0.21** |
| Globulins (g dL <sup>-1</sup> )     | 3.85 $\pm$ 0.45  | 4.13 $\pm$ 0.55  | 3.76 $\pm$ 0.26  | 4.72 $\pm$ 0.25*  |
| Creatinine (mg dL <sup>-1</sup> )   | 0.97 $\pm$ 0.18  | 1.29 $\pm$ 0.08  | 1.35 $\pm$ 0.13  | 1.35 $\pm$ 0.15   |
| BUN (mg dL <sup>-1</sup> )          | 20.41 $\pm$ 1.79 | 21.06 $\pm$ 3.86 | 19.72 $\pm$ 2.70 | 21.39 $\pm$ 2.07  |
| Phosphorus (mg dL <sup>-1</sup> )   | 5.22 $\pm$ 0.76  | 6.94 $\pm$ 1.80  | 5.32 $\pm$ 1.98  | 6.75 $\pm$ 0.49   |
| Magnesium (mg dL <sup>-1</sup> )    | 3.30 $\pm$ 0.45  | 3.28 $\pm$ 0.31  | 2.80 $\pm$ 0.24  | 2.92 $\pm$ 0.14   |

Data expressed as mean $\pm$ SD, \* $p < 0.05$ , \*\* $p < 0.01$

Table 2: Effect of clot contact time on serum biochemical parameters stored at 4°C

| Parameters                          | Hours (No = 3)   |                  |                  |                  |
|-------------------------------------|------------------|------------------|------------------|------------------|
|                                     | 0                | 3                | 6                | 24               |
| Total protein (g dL <sup>-1</sup> ) | 6.48 $\pm$ 0.44  | 7.13 $\pm$ 0.69  | 6.76 $\pm$ 0.92  | 6.26 $\pm$ 0.19  |
| Albumin (g dL <sup>-1</sup> )       | 2.63 $\pm$ 0.06  | 2.70 $\pm$ 0.08  | 2.69 $\pm$ 0.07  | 2.53 $\pm$ 0.38  |
| Globulins (g dL <sup>-1</sup> )     | 3.85 $\pm$ 0.45  | 4.43 $\pm$ 0.72  | 4.07 $\pm$ 0.99  | 3.72 $\pm$ 0.57  |
| Creatinine (mg dL <sup>-1</sup> )   | 0.97 $\pm$ 0.18  | 1.41 $\pm$ 0.13* | 1.49 $\pm$ 0.21* | 1.02 $\pm$ 0.32  |
| BUN (mg dL <sup>-1</sup> )          | 20.41 $\pm$ 1.79 | 22.17 $\pm$ 3.78 | 19.96 $\pm$ 2.94 | 20.96 $\pm$ 1.27 |
| Phosphorus (mg dL <sup>-1</sup> )   | 5.22 $\pm$ 0.76  | 6.37 $\pm$ 0.19  | 5.97 $\pm$ 0.78  | 7.47 $\pm$ 1.30* |
| Magnesium (mg dL <sup>-1</sup> )    | 3.30 $\pm$ 0.45  | 3.08 $\pm$ 1.67  | 2.67 $\pm$ 0.24  | 3.53 $\pm$ 0.28  |

Data expressed as mean $\pm$ SD, \* $p < 0.05$

The insignificant changes in serum levels of BUN and magnesium at 3, 6 and 24 h when compared with 0 h group agreed with Ehsani *et al.* (2008). Increase clot contact time resulted in significant increase in serum phosphorus after storage for 24 h in the refrigerator, significant increase in serum creatinine after 3 and 6 h at 4°C. Carothers *et al.* (1976) recommend that serum be separated from the clot within 3 h of blood collection and stored at room temperature if inorganic phosphorus concentrations are to be valid. Ono *et al.* (1981) recommend that blood can be stored at 4°C for as long as 48 h without affecting inorganic phosphorus values. According to the current study, serum phosphorus in none separated serum is stable at room temperature for 24 h and at the refrigerator for 6 h. It had been suggested that the significant increase in creatinine concentration during storage is due to non-specific formation of pseudocreatinines with kinetic jaffe reaction (Heins *et al.*, 1995).

## CONCLUSION

In this study, serum samples for total protein, BUN and magnesium can be harvested after 24 h storage at room temperature or refrigerator. For creatinine serum must be harvested before 3 h storage at 4°C, for albumin and globulins before 24 h at room temperature.

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