Correlation of Vitreous Calcium, Sodium and Glucose Levels with Postmortem Interval and Temperature in Dogs

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Abstract

The present study was undertaken to find out the correlation between increasing postmortem interval (PMI) and levels of various vitreous biochemical parameters. The vitreous humour samples were collected immediately after death from eight canine carcasses brought for necropsy. Eyes were enucleated as soon as possible after receiving carcass for postmortem. Randomly one eye, either left or right was maintained at ambient room temperature while another at refrigeration temperature (4-5°C). Sampling of vitreous humour was done at 1-4, 12, 24, 36, 48, 60, 72 and 84 hours after death. In the present study, vitreous sodium concentration did not show any significant difference between the PMI in the eyes maintained at both temperatures. A significant (P<0.05) decrease in the vitreous concentration of glucose was observed at 12 and 24 h as compared to the initial concentration at 1-4 h in the eyes maintained at normal ambient temperature while in the case of eyes maintained at refrigeration temperature no significant difference was observed between all PMI. A significant (P<0.05) decrease in the vitreous calcium concentration was observed at 48 and 60 h as compared to the initial concentration (1-4 h) at both temperatures while in the case of eyes maintained at refrigeration temperature significant (P<0.05) decrease was observed at 48, 60 and 72 h as compared to the initial concentration at 1-4 h. The vitreous calcium concentration showed significant (P<0.05) negative correlation (up to 72 h) with PMI at both temperatures while vitreous sodium and glucose concentration showed no significant correlation with PMI at both temperatures. The present study revealed that there was a linear decrease in the vitreous calcium level after death which can be used to estimate the time of death in dogs adjunct with other routinely used methods while sodium and glucose fluctuated inconsistently with no apparent relation to the time or temperature.

Key Words: Dog, Post Mortem Interval, Vitreous Calcium, Vitreous Sodium, Vitreous Glucose.

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References


Many chemical changes begin to take place in the body immediately or shortly after death and progress in a fairly orderly fashion until the body disintegrates. Each change has its own time factor or rate. These changes occur in various body fluids viz. blood, spinal fluid and vitreous humor of eye. Thus, determination of the chemical abnormalities could help forensic pathologists to ascertain time since death (TSD) more precisely (Agrawal et al., 1983).

Among various body fluids available for biochemical examination, the vitreous humor is preferred in determining time of death because it has larger volume, easily obtainable and is usually free of contamination. It is relatively inert and slightly influenced by sudden fluctuations in the blood chemistry. Accurate estimation of postmortem interval (PMI) has great values to criminal investigation and trial. The levels of chemical components in vitreous humor changes with time after death, which can help estimate the PMI (Chen et al., 2009). The present work was undertaken to study the correlation between increasing PMI and vitreous calcium, sodium and glucose levels and effect of surrounding temperature on it.

Materials and Methods
Eight canine carcasses brought for necropsy formed the material for collection of vitreous humour in the present study. The information regarding time of the death was gathered from the dog owners, clinicians and clinical records.

Eyes were enucleated as soon as possible after receiving the carcass for postmortem. Randomly one eye, either left or right was maintained at ambient room temperature while another at refrigeration temperature (4-5°C). Sampling of vitreous humor was done using 16 g needle and 20 mL syringe. The needle was inserted at the lateral angle of the eyeball so that the tip of the needle was placed at the centre of the eyeball. About 50 µL vitreous humour was gently aspirated from each eye and transferred to eppendorf tubes. The needle was left in situ till the end of the experiment. Sampling of the vitreous humour was done at 1-4, 12, 24, 36, 48, 60, 72 and 84 h after death. The aspirated vitreous humour samples were centrifuged at 13,000 rpm for 15 min at 5°C temperature and the supernatant was used for analysis. The eyes showing extensive autolysis and putrefaction were discarded and the sampling from such eyes was stopped immediately.

The estimation of the vitreous calcium, sodium and glucose concentrations was carried out immediately after collection on semi-autoanalyzer (MISHPHA BT-320) using ready-made kits (Span Diagnostics Limited, Surat, India). The results were subjected to statistical analysis by using IBM SPSS Statistics (version

### Table I. Mean (±S.E.) of calcium, sodium and glucose concentrations in vitreous humour of eyes kept at Normal Ambient Temperature (NT) at different Postmortem Intervals (PMI).

<table>
<thead>
<tr>
<th>PMI (hours)</th>
<th>Calcium (mg/dL)</th>
<th>Sodium (mEq/L)</th>
<th>Glucose (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-4</td>
<td>9.51±0.26</td>
<td>146.85±3.42</td>
<td>21.55±5.73</td>
</tr>
<tr>
<td>12</td>
<td>8.55±0.46</td>
<td>183.80±13.08</td>
<td>6.79±0.72</td>
</tr>
<tr>
<td>24</td>
<td>8.09±0.46</td>
<td>177.96±18.78</td>
<td>6.45±0.49</td>
</tr>
<tr>
<td>36</td>
<td>8.49±0.69</td>
<td>178.11±10.93</td>
<td>8.01±1.07</td>
</tr>
<tr>
<td>48</td>
<td>6.79±0.50</td>
<td>170.30±2.09</td>
<td>8.96±0.91</td>
</tr>
<tr>
<td>60</td>
<td>6.40±1.30</td>
<td>166.43±11.02</td>
<td>10.00±1.04</td>
</tr>
<tr>
<td>72</td>
<td>6.59±0.52</td>
<td>170.30±23.01</td>
<td>11.94±0.60</td>
</tr>
<tr>
<td>84</td>
<td>8.93</td>
<td>196.39</td>
<td>12.24</td>
</tr>
<tr>
<td>Pearson Correlation</td>
<td>Significant negative correlation up to 72 hours (P&lt;0.01) (r= - 0.927)</td>
<td>No significant correlation up to 72 hours (P&gt;0.05)</td>
<td>No significant correlation up to 72 hours (P&gt;0.05) (r= - 0.249)</td>
</tr>
</tbody>
</table>

Means with different superscripts within column differs significantly (P<0.05) (n=8).
The data were processed for Karl Pearson Correlation, One Way ANOVA and paired t-test.

Results and Discussion
The mean (±S.E.) concentrations of calcium, sodium and glucose at the normal and refrigeration temperature in the vitreous humour at different PMI are shown in Tables I and II. There was a significant (P<0.05) decrease in the vitreous calcium concentration at 48 and 60 hours as compared to the initial concentration (1-4 hours) at both temperatures while in the case of eyes maintained at refrigeration temperature there was a significant (P<0.05) decrease at 48, 60 and 72 h as compared to the initial concentration (1-4 h). In the present study, vitreous sodium concentration did not show any significant difference among all postmortem intervals in the eyes maintained at both temperatures. Significant (P<0.05) decrease in the vitreous concentration of glucose was observed at 12 and 24 h as compared to the initial concentration at 1-4 h in the eyes maintained at normal ambient temperature while in the case of eyes maintained at refrigeration temperature no significant difference was observed among all postmortem intervals.

Although, there was no significant difference observed among the mean values of calcium, sodium and glucose levels in the eyes maintained at normal and refrigeration temperatures at respective postmortem intervals, the calcium levels were found least affected by surrounding temperature fluctuations (Table III & Fig. 1).

The vitreous calcium concentration showed significant negative correlation (up to 72 h) with PMI at both temperatures while vitreous sodium and glucose concentration did not show any significant correlation with PMI at both temperatures.

The significant negative correlation was observed between vitreous calcium level and

<table>
<thead>
<tr>
<th>Table II</th>
<th>Mean (±S.E.) of calcium, sodium and glucose concentrations in vitreous humour of eyes kept at Refrigeration Temperature (RT) at different Postmortem Intervals (PMI).</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMI (hours)</td>
<td>Calcium (mg/dL)</td>
</tr>
<tr>
<td>-----------</td>
<td>-----------------</td>
</tr>
<tr>
<td>1-4</td>
<td>9.53±0.33</td>
</tr>
<tr>
<td>12</td>
<td>9.48±0.31</td>
</tr>
<tr>
<td>24</td>
<td>8.80±0.50</td>
</tr>
<tr>
<td>36</td>
<td>8.69±0.53</td>
</tr>
<tr>
<td>48</td>
<td>6.79±0.37</td>
</tr>
<tr>
<td>60</td>
<td>6.70±0.75</td>
</tr>
<tr>
<td>72</td>
<td>6.59±1.02</td>
</tr>
<tr>
<td>84</td>
<td>9.49</td>
</tr>
</tbody>
</table>

Pearson Correlation

Significant negative correlation up to 72 hours (p<0.01) (r= - 0.950)

No significant correlation up to 72 hours (P>0.05) (r= 0.135)

Means with different superscripts within column differs significantly (P<0.05) (n=8)

![Fig. 1. Effect of temperature on vitreous calcium concentration. (NT- Normal Ambient Temperature, RT- Refrigeration Temperature).](image-url)
PMI which is supported by previous workers (McLaughlin and McLaughlin, 1988; Mulla et al., 2005; Wilkie and Bellamy, 1982).

The findings about variation in the levels of calcium, sodium and glucose at different postmortem intervals in present study are in concordance with the earlier reports on postmortem biochemical changes in the vitreous humour (Chandrakanth et al., 2013, Brzezinski and Godlewski, 2004; Hanna et al. 1990; Mulla et al., loc. cit.; Schoning and Strafuss, 1980a). These investigators reported inconsistent fluctuations in the levels of sodium and glucose with no apparent relation with time or temperature. The fluctuation in the mean values of sodium are thought to be due to individual variation rather than postmortem change while the vitreous glucose level dropped to less than half of the antemortem value within 3 h after death with no apparent relation with either time or temperature. Furthermore, Coe (1972) stated that the continuous decrease of the glucose concentration over the postmortem interval (due to continued utilization) severely limited its use for estimating antemortem serum glucose levels within a specific postmortem time interval. The samples would have to be taken and analyzed within a short period after death. Because glucose values have been steadily falling after death, it would be unlikely to have a falsely elevated glucose level; however a low glucose level would have to be interpreted with caution.

The effect of surrounding temperature on postmortem vitreous biochemical changes in the present study are consistent with several earlier studies (McLaughlin and McLaughlin, loc. cit.; Mulla et al., loc. cit.; Schoning and Strafuss 1980b). McLaughlin and McLaughlin (loc. cit.) reported that the environmental temperature and incubation time were important variables in the interpretation of postmortem vitreous sample values. Lower temperatures and shorter incubation intervals tended to result in vitreous chemical values that varied little from fresh vitreous values. At higher temperatures and longer incubation intervals the incubated vitreous chemical values varied widely. The potassium concentration in the ocular fluids rises with an increase in the environmental temperature.

**Summary**

Vitreous humour studies in dead dogs showed a significant (P<0.05) decrease in the calcium concentration at postmortem intervals of 48 and 60 h as compared to the initial concentration (1-4 h) at both temperatures while in the case of eyes maintained at refrigeration temperature significant (P<0.05) decrease was observed at postmortem intervals 48, 60 and 72 h as compared to the initial concentration at 1-4 h. The vitreous calcium concentration showed significant (P<0.05) negative correlation (up to 72 h) with PMI at both temperatures while vitreous sodium and glucose concentration showed no significant correlation with PMI at both temperatures. All parameters studied were found unaffected by surrounding temperature.
There was a linear decrease in the vitreous calcium level after death which can be used to estimate time of death in dogs adjunct with other routinely used methods while sodium and glucose fluctuated inconsistently with no apparent relation to the time or temperature.

References


Abstract

This biological experiment was carried out to study the role of chelated minerals in White Leghorn layer diets on egg production performance from 41 to 72 weeks. The basal layer diet (T₃) was formulated as per the standards prescribed in Bureau of Indian Standards (B.I.S, 1992) except for the copper, manganese and zinc levels. T₂ and T₄ diets were prepared by incorporating inorganic and organic Cu, Mn and Zn along with basal diets, respectively to meet out the B.I.S recommendations for Cu, Mn and Zn. T₄, T₅ and T₆ diets contained 50, 100 and 150 per cent more levels of Cu, Mn and Zn, respectively by adding inorganic minerals. Similarly, T₇, T₈ and T₉ diets were prepared with organic minerals as that of T₄, T₅ and T₆. It could be concluded that BIS with organic Cu, Mn and Zn (T₇) and BIS with 50 per cent higher organic Cu, Mn and Zn (T₈) in layer diets recorded significantly