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Lack of Correlation of Postmortem Vitreous Humor Calcium Concentration with Antemortem Serum Calcium Concentration

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ABSTRACT: Although results of several studies measuring calcium concentrations in vitreous humor have been published, comparisons of postmortem vitreous calcium with antemortem serum calcium have not been made. In a study of 30 cases in which antemortem calcium results were available, the mean vitreous calcium concentration was 6.8 mg/dL. There was no significant change in vitreous calcium postmortem nor significant correlation between vitreous calcium and antemortem serum calcium. Experimental observations suggest that vitreous calcium is regulated by an active transport mechanism. Vitreous calcium measurements do not appear to be useful in evaluating suspected antemortem abnormalities of serum calcium.

KEYWORDS: pathology and biology, postmortem examinations, vitreous humor, calcium, metabolism, serum

Disorders of calcium metabolism are relatively common in terminally ill patients. In a recent study at the Naval Regional Medical Center, Portsmouth, VA, either hypercalcemia or hypocalcemia was present in 17 of 76 consecutive cases of autopsied individuals in whom antemortem serum calcium concentrations were determined. Parathyroid gland abnormalities were present in an additional seven of the individuals with normal serum calcium [*1*]. Thus, almost one third of the 76 cases had abnormalities in which postmortem determination of calcium concentration might be of value in the interpretation of anatomic and clinical findings.

The literature on the usefulness of postmortem calcium determination is scant. Hodgkinson and Hambleton [*2*] showed that serum calcium rises rapidly after death, apparently because of a loss of water from plasma to cells. Thus, postmortem measurement of serum calcium is not useful. On the other hand, postmortem changes in vitreous calcium concentration occur slowly, if at all. Coe [*3*], Bito and Salvador [*4*], and Blumenfeld et al [*5*] found no significant change in vitreous calcium concentration with increasing postmortem interval. In addition, Swift et al [*6*] evaluated postmortem changes in vitreous calcium in 20 children

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by sampling fluid from the two eyes at different postmortem intervals. In ten of the cases, the calcium concentration was higher in the second specimen and in the other ten it was higher in the first specimen. In some cases, the difference between two samples taken 24 h apart was as much as 2 mg/dL. This variability raises the question of how well the vitreous calcium concentration correlates with a patient's antemortem calcium concentration.

Most previous studies have not compared vitreous calcium with antemortem serum calcium concentrations. Although Swift et al [*6*] determined vitreous calcium in 43 children, only five had antemortem calcium levels available for comparison. In these five cases, the difference between serum and vitreous calcium ranged between −0.7 and 2.4 mg/dL. Sturner et al [*7*] found several instances in which vitreous calcium was unexpectedly low in previously healthy patients who had died accidentally. Coe [*3*] found that vitreous calcium was normal in hypocalcemic individuals and concluded that there is no evidence that antemortem calcium abnormalities can be detected by measuring vitreous calcium [*8*]. As part of a study of disorders of the parathyroid gland at autopsy, vitreous calcium was determined in 30 cases. This study reports a comparison between antemortem serum calcium and vitreous calcium in these 30 cases.

Materials and Methods

The 30 cases forming this study were autopsied over a 15-month period. In all cases vitreous fluid was collected with a 21-gauge needle by a single insertion near the the limbus on the lateral aspect of each eye, and the fluid from the two eyes was pooled; from 2 to 3 cm^3 total vitreous fluid was obtained. Only clear fluid was used for analysis; opaque or cloudy fluid was discarded. Calcium concentration in the uncentrifuged fluid was determined using a modified cresolphthalein/sodium edetate procedure with 8-hydroxyquinolol to reduce magnesium interference. All tests were performed on the automatic clinical analyzer (ACA) (Du Pont). This method has been reported to have a maximum positive interference of 0.5 mg/dL at magnesium concentrations of 7 mEq/L and no interference from magnesium at normal serum concentrations (Du Pont Laboratories).

The cause of death included malignancy in 12 cases, chronic renal failure in 3 cases, and medical or surgical disorders in the remaining 15 cases. Results of antemortem calcium and albumin measured on the sequential multiple analysis computer (Technicon Instruments) were available in all cases. Calcium is determined on this instrument by a method similar to that of the ACA using cresolphthalein/sodium edetate and 8-hydroxyquinolol; parallel studies in this laboratory showed slightly lower values on the ACA ($y = 0.995x - 0.393$, $r = 0.9925$) but no proportional bias between the two instruments. In cases in which multiple values were recorded, the one obtained closest to death was used for correlation. In all but five of the 30 cases, serum calcium concentration was measured within 48 h of death. Adjustment of serum calcium for low serum albumin was made using the formula of Payne et al [*9*]:

$$\text{adjusted calcium} = \text{serum calcium (mg/dL)} - \text{serum albumin (g/dL)} + 4.0$$

Correlation was evaluated by using linear regression analysis by least squares. Differences in distribution of values between groups were evaluated with the Mann-Whitney U test.

Results and Discussion

A brief summary of the causes of death, postmortem interval, antemortem laboratory results, and vitreous calcium in each case is given in Table 1. The mean vitreous calcium concentration was 6.9 mg/dL, with a range from 5.7 to 8.4 mg/dL. There was no significant change in calcium with increasing postmortem interval ($r = 0.254$, $p > 0.1$); the slope of the regression line was nearly horizontal (0.013 mg/dL · h^{-1}).

TABLE 1—*Calcium concentrations measured in 30 cases.*

Case	Cause of Death	Postmortem Interval, h	Serum Calcium, mg/dL	Adjusted Serum Calcium, mg/dL	Vitreous Calcium, mg/dL
1	hepatoma	21	8.7	10.3	5.7
2	melanoma	13	7.7	9.7	6.3
3	congestive heart failure	3	8.4	9.8	7.5
4	ovarian carcinoma	7	7.1	9.1	6.3
5	postoperative complications	1	9.1	9.8	7.2
6	myocardial infarct	11	9.3	9.6	6.8
7	cerebrovascular accident	1	8.5	9.3	7.3
8	cirrhosis	20	8.6	10.4	6.9
9	lung cancer	14	8.3	10.1	6.8
10	lung cancer	16	7.2	9.2	6.7
11	cholangiocarcinoma	54	9.0	9.6	6.8
12	postoperative complications	12	9.4	10.0	7.2
13	lymphoma	24	7.2	9.3	8.0
14	coronary atherosclerosis	25	9.7	9.2	6.3
15	palatal carcinoma	4	9.5	11.0	7.2
16	cirrhosis	20	9.1	10.0	7.3
17	uterine rupture	39	8.9	9.7	7.4
18	brain abscess	23	9.9	9.5	8.4
19	colon carcinoma	30	8.3	10.3	7.2
20	Wegener's granulomatosis	3	8.0	9.6	6.3
21	pulmonary embolism	8	9.1	8.9	6.5
22	myxedema	14	7.4	9.6	6.6
23	ruptured berry aneurysm	17	9.2	9.5	7.5
24	chronic renal failure	6	6.3	7.6	5.9
25	lung carcinoma	19	9.9	9.5	7.1
26	chronic renal failure	21	7.4	8.7	7.1
27	pneumonia	8	7.2	8.3	6.2
28	gastric carcinoma	4	8.3	10.4	6.4
29	lung carcinoma	54	9.3	10.4	7.2
30	postoperative complications	16	9.8	9.5	6.0

This finding is similar to the observations of other researchers [*3–6, 10*], who found no significant postmortem change in vitreous calcium. The mean vitreous calcium concentration of 6.9 mg/dL is similar to Coe's mean value [*3*] of 6.8 mg/dL and only slightly lower than Naumann's mean [*10*] of 7.2 mg/dL. It would seem that the method used for measuring calcium concentration is not critical, as similar results for mean and postmortem stability have been reported using cresolphthalein/sodium edetate [*3*], atomic absorption [*4–6*], and the Clark-Collip oxalate precipitation method [*10*]. Although Coe [*8*] suggested that cresolphthalein would not be an acceptable method because of interference by magnesium, his results and those of Sturner et al [*7*] and this study do not support his conclusion. Vitreous magnesium concentration does not change significantly after death [*4–6*], and the concentration of magnesium observed is not associated with interference in the cresolphthalein method for calcium used in most current procedures.

The relationship between postmortem vitreous calcium and antemortem serum calcium was evaluated in several ways. Serum calcium was higher than vitreous calcium in 29 of the 30 cases; the mean difference was 1.7 mg/dL, with a range of −0.8 to 3.8 mg/dL. As shown in Fig. 1, the correlation between vitreous and serum calcium was poor and did not achieve statistical significance ($r = 0.312$, $p > 0.05$). Vitreous calcium was somewhat lower in the 13 hypocalcemic individuals (serum calcium less than 8.6 mg/dL), with a mean of 6.7 mg/dL compared to 7.0 mg/dL in the 17 individuals with normal calcium concentrations.

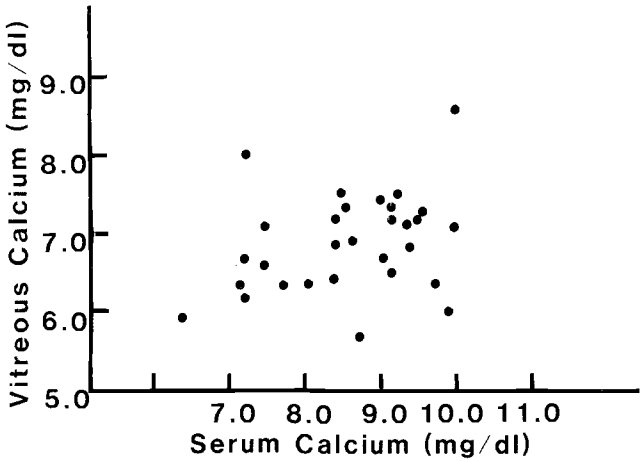

FIG. 1—*Postmortem vitreous calcium concentration compared with antemortem serum calcium concentration. The graph reveals the poor correlation observed between the two measurements (y = 0.23x + 5.05, r = 0.312).*

While this difference is statistically significant ($U = 56$, $p < 0.025$), the range of values made it impossible to identify hypocalcemic patients by using vitreous calcium.

Since decreased serum albumin concentration results in decreased total calcium without changing ionized or diffusable calcium, total calcium was adjusted for hypoalbuminemia using the formula of Payne et al [*9*]. If vitreous calcium were related to ionized or diffusable serum calcium, the adjustment should strengthen the correlation between vitreous and serum calcium. In fact, there was no correlation between vitreous and adjusted serum calcium ($r = 0.253$, $P > 0.1$), and the mean vitreous calcium was identical in cases with adjusted serum calcium concentrations over or under 9.5 mg/dL.

This lack of correlation between serum and vitreous calcium was expected, based on observations in a small number of cases by Coe [*3*], Swift et al [*6*], and Sturner et al [*7*]. Another observation suggesting that vitreous calcium is not directly related to serum calcium is that the observed mean vitreous calcium (approximately 7 mg/dL) is intermediate between dialyzable serum calcium (approximately 5.2 mg/dL) and total serum calcium (approximately 9.5 mg/dL). Bito [*11*] made careful studies in several animal species and found a similar disparity between dialyzable serum calcium and vitreous calcium. By dividing the vitreous body in half and collecting fluid from each half separately, he also found a concentration gradient from the posterior to the anterior vitreous fluids. Bito concluded that calcium concentration in the vitreous humor is regulated by an active transport mechanism. This hypothesis explains both the observed levels of calcium in the vitreous fluid and the lack of correlation between serum and vitreous calcium concentrations. Active transport mechanisms are able to maintain levels of a given substance within a fluid compartment even against a concentration gradient, so that low serum levels do not necessarily result in lowered concentration of an actively transported molecule. Also, since transport mechanisms may become saturated, elevated serum levels may not have a marked effect on concentration in the isolated compartment.

If, as the observations suggest, vitreous calcium is regulated by an active transport mechanism, it would not be logical to attempt to predict serum calcium by measuring the concentration of calcium in the vitreous fluid. Thus, although vitreous calcium is stable in the postmortem period, it would appear that Coe is correct in stating that serum calcium abnormalities cannot be evaluated by measuring the vitreous calcium concentration.

Summary

1. The vitreous calcium concentration does not change significantly following death.

2. The vitreous calcium concentration is usually between 6.0 and 8.0 mg/dL; this value is intermediate between dialyzable serum calcium (approximately 5.2 mg/dL) and total serum calcium (approximately 9.5 mg/dL), suggesting an active transport of calcium.

3. There is a poor correlation between serum and vitreous calcium concentrations, probably because of this active transport mechanism.

4. Measurement of vitreous calcium concentration is not useful for evaluating antemortem serum calcium concentration.

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