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Comparison of Ante- and Post-Mortem PSA Levels for Epidemiological Studies

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Abstract. *Background. Valuable correlations could be made between serum prostate specific antigen (PSA) and prostate histopathology by the use of autopsy sampling if post-mortem PSA data were informative. However, PSA forms and levels in autopsy sera have not been investigated. Materials and Methods: Paired ante- and post-mortem sera were collected for a series of cases. Total and free PSA levels in each were determined and compared. These PSA data were correlated with corresponding changes in serum electrolyte levels. Results: Total PSA levels were similar in ante- and post-mortem sera if autopsy blood was drawn by ~24 hours following the time of death. Free PSA levels, however, were increased approximately two-fold in most post-mortem sera analyzed. Increases in the serum electrolytes potassium, magnesium and phosphate correlated positively with increases in free PSA. Conclusion: Total PSA levels in ante- and post-mortem sera were comparable. Free PSA levels had approximately doubled by autopsy, but may be normalized in relation to increases in serum electrolyte levels. The use of autopsy prostates and PSA data would avoid diagnostic bias from use of clinical material, and permit extensive analysis to be carried out, which is not normally feasible with live subjects.*

The levels of PSA in serum now play a key role in the detection of prostate cancer (PCa). In combination with digital rectal exam and imaging studies, elevated PSA is an important indicator for further diagnostic workup, such as biopsying the prostate. Age-indexed thresholds of total PSA have been established by Partin and coworkers for evaluating probable risk for prostate cancer (1), although pathologies other than PCa such as prostatitis are also associated with elevated PSA. It is also recognized that relative levels of free PSA are lower for PCa than other prostate diseases (2, 3). The use of autopsy material would allow for comprehensive analysis of prostate histopathology and PSA forms and levels, but the reliability of autopsy PSA data has not been evaluated. The present study evaluated the levels of total and free PSA in autopsy serum relative to ante-mortem levels so that such systematic correlations with prostate lesions can be made.

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Materials and Methods

Patient confidentiality. Original case information and all patient identifiers were replaced with anonymous labels for this study once ante- and post-mortem samples were paired.

Preparation of serum. Ante- and post-mortem sera for this study were provided by the Syracuse, NY and Albany, NY VA Medical Centers, and SUNY Upstate Medical University/Onondaga County Medical Examiners Office in Syracuse, NY, USA. The study was approved by the Institutional Review Boards of those institutions. Ante-mortem sera prepared earlier for purposes of patient care, but to be discarded following demise, were made available to this study. The sera were acceptable if they had been prepared less than a week prior to the patient's death and stored at 5˚C or frozen. Post-mortem blood was drawn at autopsy either from the femoral vein or by intracardiac puncture, usually less than 24 hours following the time of death. "Red Top" or "Gold Top" tubes (Becton Dickinson, BD Biosciences, Bedford, MA, USA) were used for blood collection, which are designed for this purpose. Usually 5 to 10 ml of blood was obtained. Serum was prepared by conventional means by low-speed centrifugation (1800 x g for 15 minutes) and stored frozen at -20˚C. Prior to assay for PSA, autopsy sera were clarified by recentrifugation at ~200,000 xg for 10 minutes in a Beckman Airfuge (Beckman Instruments, Fullerton, CA, USA). This helped eliminate assay background caused by cellular contaminants in many post-mortem serum samples.

PSA determinations. Total PSA levels were determined initially by the Syracuse, NY VA Medical Center Clinical Laboratory using an ELISA-based immunodetection method (Axsym, Abbott Laboratories, Abbott Park, IL, USA). Subsequently, total and free PSA determinations were performed with an immunochemoluminescence method (Access Immunoassay System, Beckman Coulter, Fullerton,

Table I. *Effects of ultracentrifugation and hemolysis on PSA determinations.*

Expt. #	PSA-positive control (μl)	Ultracentrifugation	Hemolyzed blood (μl)	tPSA (ng.ml)[a]	% difference[b]
1	400	-	0	38.3	
2	400	+	0	37.9	-1.04%
3	200	-	200	40.2	+4.96%

[a]Values corrected for dilution
[b]% change from PSA positive control

CA, USA) by the University Hospital Clinical Laboratory, Syracuse, NY, using reagents provided by the supplier. Total PSA levels determined for ante-mortem control sera by both methods were virtually the same within experimental error (+/- ~5%).

Electrolyte determinations. Concentrations of a standard panel of serum electrolytes, including potassium, magnesium, carbonate and phosphate ($[K^+]$, $[Mg^{++}]$, $[CO_2^=]$ and $[PO_4^=]$, respectively), were determined by the Syracuse, NY VA Medical Center Clinical Laboratory using a Dade Dimension Clinical Chemistry System (Dade, Newark, DE, USA).

Results

First, it was determined that PSA values were not significantly affected by ultracentrifugation in the Beckman Airfuge used to clarify autopsy sera (see Methods and Materials). PSA-positive control serum was prepared by pooling three clinical samples with elevated PSA levels, which gave a combined value of 38.3 ng/ml total PSA (tPSA). Following ultracentrifugation, tPSA was 37.9 ng/ml, a value within experimental error (Table I). Next, the effect of hemolysis on PSA determinations was investigated, since varying degrees of hemolysis are encountered in autopsy blood samples. A blood sample from a normal male volunteer (tPSA: 0.2 ng/ml) was hemolyzed and used to prepare serum as for autopsy blood samples. The PSA-positive control was mixed 1:1 with either the hemolyzed serum or normal saline. The dilution-corrected tPSA values were the same for both samples within experimental error (<5.0%; tPSA determined with the Beckman Coulter Access system).

Total and free PSAs (tPSA and fPSA, respectively) were then determined for pairs of ante- and post-mortem sera from a series of autopsy cases (n=10). The results are shown in Figure 1. In nearly all cases, the tPSA levels were approximately the same in post-mortem sera. For the evaluable pairs of samples shown in Figure 1, the ratio of post- to ante-mortem tPSA was 0.97 (standard deviation: 0.19). In the case of fPSA, however, the values were 1.94-fold higher for this series of post-mortem sera (standard deviation: 0.69), suggesting significant dissociation of complexed PSA had occurred by the time autopsy blood was collected. In two additional cases, tPSA and fPSA for both ante-mortem and the corresponding post-mortem sera (from blood taken at ~24 hours following death) were all <0.01 ng/ml (data not shown). This indicated that the elevations in fPSA seen for the series shown in Figure 1 were not artifactually inflated values resulting, for example, from hemolysis products.

To test the effect of extended incubation at ambient temperature on PSA levels as determined by immunochemoluminescence (Beckman Coulter Access), a sample of post-mortem blood taken 21 hours following death from a 71-year-old Caucasian male was assayed immediately and again after incubation at ~25°C for 5 days. tPSA increased from 10.5 to 22.6 ng/ml; fPSA increased from 2.04 to 11.06 ng/ml (data not included in Table II). These results are in accord with studies published by Lilja and coworkers (6), demonstrating that tPSA does not decrease during incubation *in vitro* up to 1 week at either 5°C and 35°C, while complexed PSA dissociates at 35°C. The increase in tPSA seen may have resulted from dissociation of occult PSA, in which epitopes normally recognized by antibodies used for tPSA determination are occluded by complexing with alpha-2 macroglobulin.

A review of the literature on post-mortem changes indicated that, among other changes, increases in extracellular K^+ and $PO_4^=$ levels are significant (4, 5). The concentrations of these electrolytes in serum are normally tightly controlled *in vivo*. Therefore, the concentrations of a panel of serum electrolytes in pairs of ante- and post-mortem sera were determined to see if "surrogate markers" for post-mortem changes could be identified for normalization of fPSA values. As shown in Figure 2, it was found that $[K^+]$, $[Mg^{++}]$ and $[PO_4^=]$ had undergone prominent increases, and these correlated with increases in fPSA. The increases in $[K^+]$ were 5- to 6-fold, $[Mg^{++}]$ were ~2-fold higher, and $[PO_4^=]$ ~4-fold higher.

Discussion

The objective of this study was to assess the validity of tPSA and fPSA levels in autopsy sera, *i.e.*, the relationship of PSA levels in post-mortem and corresponding ante-mortem serum. For this purpose, PSA levels in ante- *vs* post-mortem

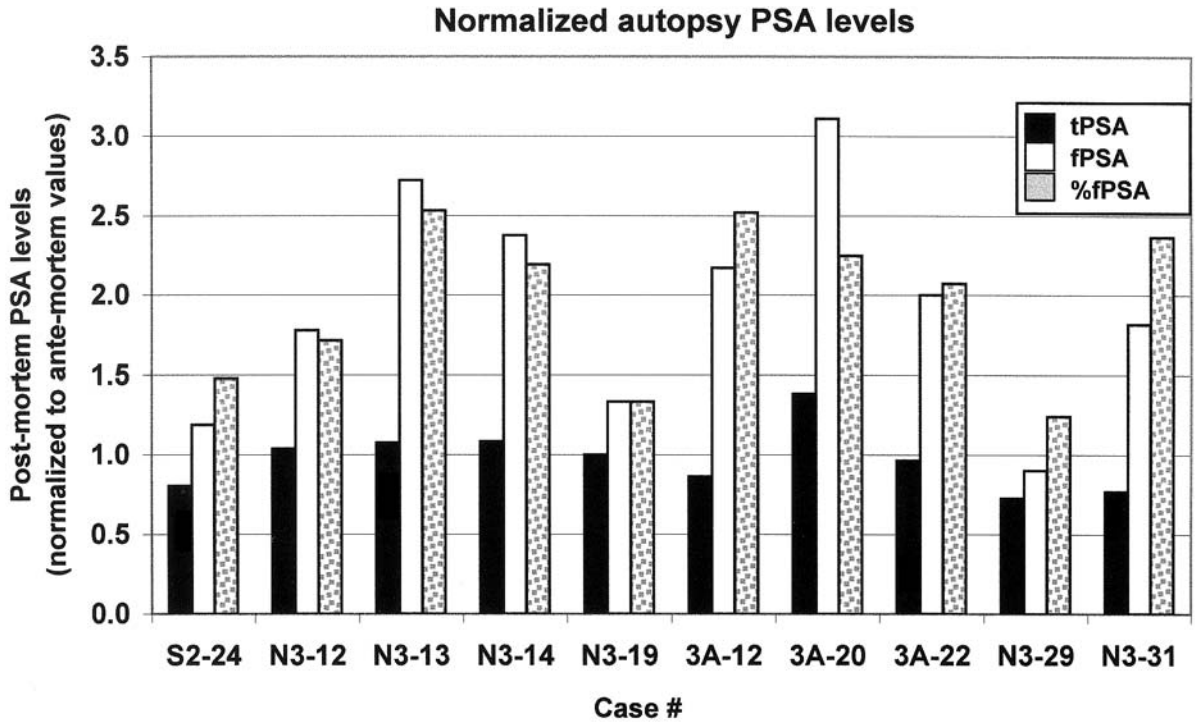

Figure 1. *Normalized post-mortem PSA data. Post-mortem tPSA, fPSA and %fPSA were normalized to ante-mortem PSA values (data shown in Table II). A value of 1 indicates that ante-and post-mortem values were the same. Most normalized post-mortem tPSA's were close to 1, while most fPSA's and %fPSA's were ~2 for the series.*

Table II. *Ratios of post-mortem to ante-mortem PSA levels.*

		PSA levels (ng/ml)			Normalized to ante-mortem data[b]		
Case #	Sample[a]	tPSA	fPSA	%fPSA	tPSA	fPSA	%fPSA
S2-24	ante	17.06	0.64	3.75			
	post	13.71	0.76	5.54	0.80	1.19	1.48
N3-12	ante	126.76	9.20	7.26			
	post	131.52	16.37	12.45	1.04	1.78	1.71
N3-13	ante	0.93	0.18	19.35			
	post	1.00	0.49	49.00	1.08	2.72	2.53
N3-14	ante	0.60	0.08	13.33			
	post	0.65	0.19	29.23	1.08	2.38	2.19
N3-19	ante	0.04	0.03	75.00			
	post	0.04	0.04	100.00	1.00	1.33	1.33
3A-12	ante	12.82	4.38	34.17			
	post	11.05	9.51	86.06	0.86	2.17	2.52
3A-20	ante	3.05	0.99	32.46			
	post	4.22	3.08	72.99	1.38	3.11	2.25
3A-22	ante	1.14	0.03	2.63			
	post	1.10	0.06	5.45	0.96	2.00	2.07
N3-29	ante	19.66	4.30	21.87			
	post	14.29	3.88	27.15	0.73	0.90	1.24
N3-31	ante	89.20	4.21	4.72			
	post	68.51	7.65	11.17	0.77	1.82	2.37

[a]ante: ante-mortem serum sample for indicated case #; post: corresponding post-mortem serum.
[b]Post-mortem PSAs normalized to corresponding ante-mortem values for each case.

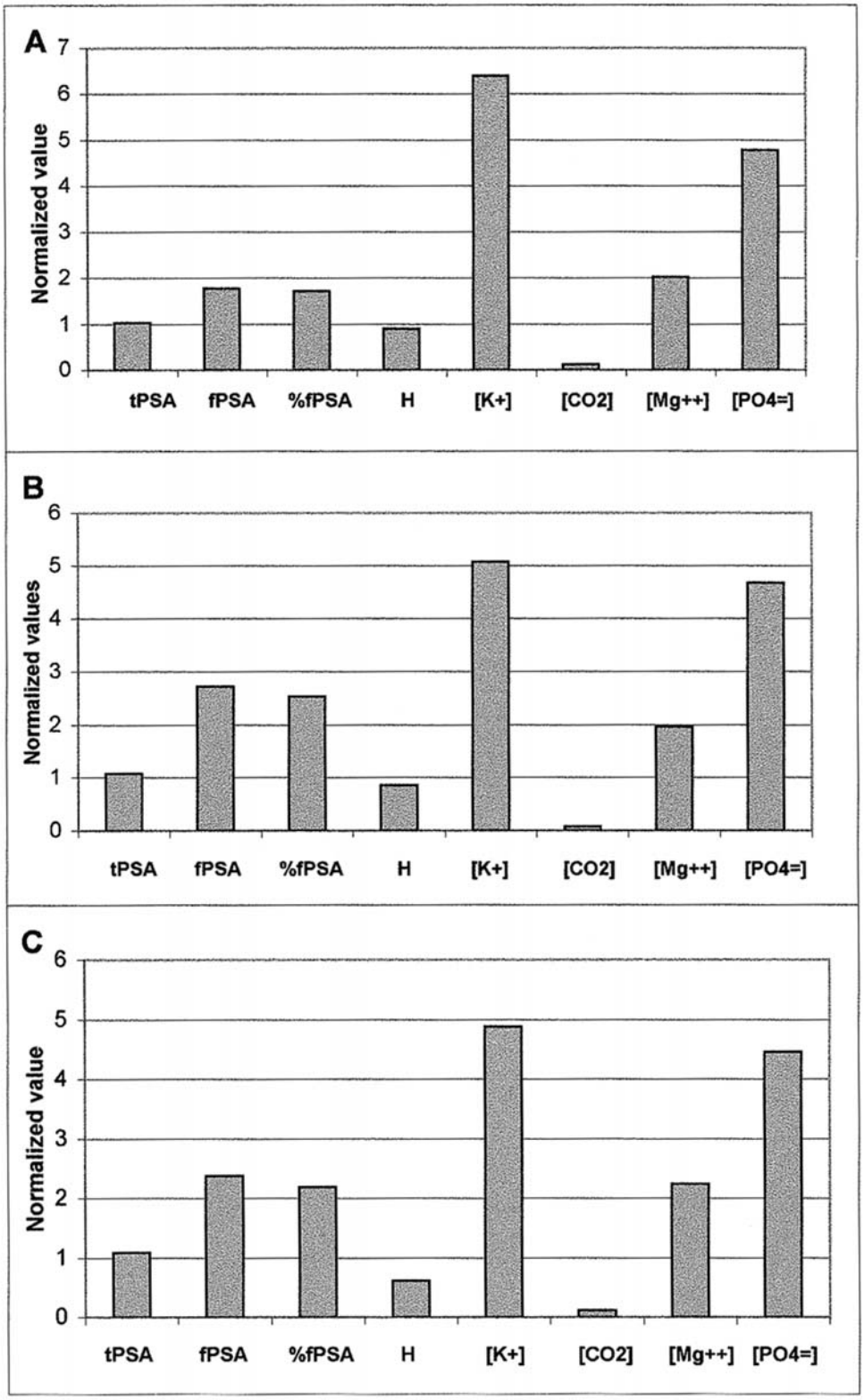

Figure 2. *Increases in selected post-mortem serum electrolyte concentrations paralleled increases in fPSA. The concentrations of K^+, Mg^{++}, $CO_2^=$ and $PO_4^=$ in post-mortem sera were normalized to ante-mortem levels for the three cases indicated in Figure 2. The autopsy times (H) were normalized to 24 hours (e.g., the H value is 1.0 when the autopsy was done 24 hours after time of death). Panel A, case N3-12; panel B, case N3-13; panel C, case N3-14. The increases in $[K^+]$, $[Mg^{++}]$ and $[PO_4^=]$ correlated positively with those of fPSA, while $[CO_2^=]$ was inversely correlated.*

sera for a series of autopsy cases were compared to determine whether PSA forms or levels undergo significant changes following death. A number of factors could potentially alter either PSA levels *per se*, or compromise PSA assays. From *in vitro* studies on PSA stability (6), it is known that the dissociation of complexed to free PSA is accelerated by alkaline pH (*e.g.*, pH 7.8 *vs* 6.2), temperature (35˚C *vs* 5˚C), and the relative molar excess of alpha1-antichymotrypsin (ACT) – with which much of total PSA is complexed in serum *in vivo*. In the present study, it was found that tPSA levels in ante- and post-mortem sera, as determined by an immunochemoluminescence method, were very similar (differing by <20% from ante-mortem values). It should be noted that for three cases assayed by an older ELISA-based method (Axsym, Abbott Laboratories), apparent declines of up to 70% in tPSA were seen in 24-hour autopsy sera, so possibly the ELISA PSA assay method is more sensitive to inhibition by post-mortem changes.

In contrast to total PSA, free PSA was increased approximately 2-fold in post-mortem sera in most cases. Since immuno-detection of fPSA is based on exposure of PSA epitopes not occluded by kallikrein protease inhibitors in circulation (most commonly alpha1-antichymotrypsin and alpha2-macroglobulin), corrections for the increases in free PSA seen could, in principle, be made based on biochemical parameters factors known to destabilize complexed or occult PSA (6). These would include how soon the decedent was transferred to the morgue, the rate of body cooling, body weight, alkalinization of blood, the site of blood collection (*e.g.*, femoral vein or intracardiac puncture) and how soon the autopsy was performed. Direct measurement of decay rates of complexed into free PSA might be undertaken for each serum sample. However, the amount of sample available is usually insufficient to carry out such studies, and the cooling rate and other factors directly controlling dissociation rates are typically not known for a given decedent or this information is unreliable. Therefore, surrogate markers for post-mortem changes were sought that could be readily determined. Increases in serum electrolytes could potentially serve this purpose. We found that the increases in serum [Mg^{++}] closely paralleled those of free PSA in most cases analyzed (Figure 2). Increases in [K^{+}], and [$PO_4^{=}$] were more prominent, ~6-fold and 4-fold, respectively. Weighted multivariate analysis of these values might provide reasonable correction of post-mortem fPSA back to time of death.

In summary, when autopsies were performed within ~24 hours following the time of death, and serum was prepared promptly, total PSA determined on post-mortem serum was found to be in reasonable agreement with ante-mortem levels in most cases analyzed. Free PSA was increased an average of ~2-fold in post-mortem sera, but was variable. However, these fPSA values might be correctable to approximate ante-mortem levels by assuming that weighted increases in serum electrolytes closely paralleled those of the dissociation of complexed PSA.

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