STABILITY OF TOTAL AND FREE PROSTATE SPECIFIC ANTIGEN IN SERUM SAMPLES AT DIFFERENT STORAGE CONDITIONS

G. Rajani Kumari and T. Malati

Department of Biochemistry, Nizam's Institute of Medical Sciences, Punjagutta, Hyderabad-500 082

ABSTRACT

The present preliminary study was performed to find out stability of total prostate specific antigen (PSA) and free prostate specific antigen (FPSA) in serum of healthy males as well as in patients of benign and malignant disorders of prostate at various freezing and nonfreezing temperatures and at different duration of time.

The results of our study indicated long-term stability of both the analytes in frozen serum. Serum total and free PSA were stable only for three to four days in regular refrigerators in unfrozen states. Clotted blood kept at room temperature (25°C-30°C) did not cause change in concentrations of both the analytes for twenty four hours.

KEY WORDS

Free PSA and Total PSA / BPH / Cancer Prostate.

INTRODUCTION

Molecular forms of prostate specific antigen (PSA) are known to circulate in blood because of complex formation with different protease inhibitors. The PSA with α_1 -antichymotrypsin complex (PSA-ACT) remains the major immunoreactive form of PSA constituting 90-95% of total PSA in serum. Other complexes of PSA with serpins (serine protease inhibitors) are PSA-AT (μ 1-antitrypsin) and PSA-PCI (protease C inhibitor). PSA-AT and PSA-PCI occur at much lower concentrations in serum. The unbound or free or noncomplex prostate specific antigen (FPSA) constitutes only about 5-15% of the total immunoreactive forms.

In the present study an attempt was made to study the effect of storage at different temperatures on free as well as total PSA in serum separated from clotted blood kept at different time and from serum immediately after separation from clotted blood. The stability of total and free PSA in sera was studied in detail for different durations after sample collection and exposing the samples at different temperatures. The detailed work up and understanding of stability characteristics of free and total PSA will finally help in specimen handling in fields and in laboratory for their correct assessment.

Address for Correspondence :

Dr. T. Malati
Department of Biochemistry,
Nizam's Institute of Medical Sciences,
Punjagutta, Hyderabad-500 082, INDIA
E-mail : malati@hd2.vsnl.net.in

The assessment of the stability of total and free PSA in serum separated from clotted blood stored for different duration without serum separation at room temperature in remote villages of India will render help in performing cancer prostate screening as a part of community health programme. We also wanted to know if blood clot in coagulated blood had any effect on total and free PSA before serum is separated.

MATERIAL AND METHODS

Stability of Total and Free PSA in coagulated blood at 2°-8°C

In order to evaluate the stability of total and free PSA six samples of blood were collected at room temperature in plain tube without any anticoagulant from 65 year old male patient of BPH (sample A) and from 60 year old male patient of cancer prostate (sample B). All aliquots were kept at room temperature for different duration ranging from 1 hour to 24 hours. Serum was separated from clot at different time intervals. However, after separation of clear serum, all aliquots were stored at 2-8°C in regular refrigerators for two days and total and free PSA were estimated using streptavidin biotin immunoassay technology of Boehringer Mannheim, Germany.

Stability of total and free PSA in serum at 2-8°C

Blood was collected from a healthy male, two patients of BPH and two patients of carcinoma prostate. The samples were allowed to clot at room

temperature for half an hour and serum was separated immediately after clot retraction. Six aliquots of each sample were made and stored at 2-8°C for one, four, nine, twelve, fourteen and twenty five days.

Stability of total and free PSA at -8 to -10°C

Blood was collected from a 60 year old male patient of adenocarcinoma prostate at regression stage of disease. Serum was separated immediately after clot retraction. Several aliquots of sera were made and stored frozen at -8 to -10°C. Total and Free PSA was measured at different time intervals.

RESULTS

Stability of total and free PSA in coagulated blood at 2-8°C (Table 1)

Total and free PSA were quite stable for 24 hours at 25° to 30°C hence we were quite sure that transportation of clotted blood sample from primary health care centers in remote villages to tertiary center like ours will not have any effect on concentration of total and free serum PSA. The total PSA at base line in serum separated without storage of whole blood revealed a concentration of 4.7 ng/ml. The values ranged from 4.7 ng/ml to 5.6 ng/ml at different duration from 1 to 24 hours. Irrespective of source, the free PSA was also stable at different duration of intervals. The values varied from 13.0 ng/ml to 14.0 ng/ml within 24 hours.

Table 1. Evaluation of stability of total and free PSA in coagulated blood at 2-8°C

Duration of serum separation from clot (hours)	Total PSA ng/ml (Patient A)	Free PSA ng/ml (Patient B)
0	4.7	13.0
1	4.8	13.0
2	4.9	13.7
5	5.0	13.0
10	5.2	14.3
24	5.6	14.2
Coefficient variation	6.36	4.59

Stability of total and free PSA in serum at 2-8°C (Table 2)

The concentration of total and free PSA in different sera declined progressively, when stored unfrozen

in regular refrigerators between 2-8°C. The reduction in the concentration of total and free PSA was progressive from 4th day onwards till 25th day of storage, irrespective of the fact whether the samples were collected from healthy individuals or from patients of BPH or cancer prostate. However, the free PSA in normal healthy serum showed declining concentrations beyond 9th day. PSA concentration decreased from 100% to 57% on 12th day and 25th day respectively. Both sera from Ca. Prostate patient showed declining concentration of free PSA from 8.7%-22% on fourth day to 59%-72% on fourteenth day. Surprisingly, only in one patient diagnosed as cancer prostate, a very high total PSA of 3600 ng/ml did not have the expected decrease of total PSA concentration (ranged from 3600 ng/ml to 3450 ng/ml). However, the corresponding free PSA of 400 ng/ml had progressive decline from 4th day onward till 14th day (22% to 72%). In both patients of BPH, the decline in concentration of total PSA ranged from 21%-44% at fourth day to 55%-82% on 25th day. The decline of total PSA was very progressive from 10% to 86% in one patient of carcinoma prostate. The free PSA concentration decreased from 17%-28% on 4th day to 86%-100% on 25th day in both cases of BPH.

Stability of total and free PSA at -8 to -10°C (Table 3)

The results of total and free PSA stability in frozen sera are summarized in Table 3. It was interesting to note that both free and total PSA were stable for more than four months (128 days) after storage at -8 to -10°C. There was no change in total PSA concentration on 110th day (24 ng/ml) compared to its concentration (24 ng/ml) on 0 day. At 128th day the concentration of total PSA declined very little to 21 ng/ml. The free PSA value was 5.1 ng/ml on 70th day compared to 5.2 ng/ml on 0 day. Its concentration declined to 4.1 ng/ml on 128th day.

DISCUSSION

The study on the stability of both total PSA and free PSA in serum from healthy males as well as patients of BPH and carcinoma prostate plays a very important role in establishing the guidelines for blood collection, serum separation and storage of the serum samples at required temperatures for the correct assessment of both the analytes. Additionally it was important to study their stability if the blood was collected at room temperature in remote villages of India for cancer prostate screening as a part of community health program. It was necessary to know if coagulated blood had any effect on total and free PSA concentration before the serum was separated.

Table 2. Stability of total and free PSA in serum at 2-8°C

	1 st Day	4 th Day	9 th Day	12 th Day	14 th Day	25 th Day
TOTAL PSA (ng/ml)						
Healthy male	0.7	0.4	0.48	0.28	0.22	0.2
(% Decrease)		(43)	(31.4)	(60)	(68)	(71)
BPH I	5.7	3.2	2.0	1.9	1.6	1.0
(% Decrease)		(44)	(65)	(67)	(72)	(82)
BPH II	14.1	11.1	10.9	7.5	6.5	6.3
(% Decrease)		(21)	(22)	(47)	(54)	(55)
Ca. Prostate I	42.5	38.0	34.0	15.1	8.4	5.8
(% Decrease)		(10)	(20)	(64)	(80)	(86)
Ca. Prostate II	3600	3400	3600	3200	3450	—
FREE PSA (ng/ml)						
Healthy male	0.4	0.4	0.4	0.17	0.15	0.0
(% Decrease)		(Nil)	(Nil)	(57)	(63)	(100)
BPH I	2.17	1.8	1.26	1.4	1.2	0.3
(% Decrease)		(17)	(42)	(35)	(44)	(86)
BPH II	4.9	3.5	2.7	1.6	1.2	0.0
(% Decrease)		(28)	(45)	(67)	(75)	(100)
Ca. Prostate I	16.0	14.6	15.9	9.0	6.6	5.3
(% Decrease)		(8.7)	(13)	(44)	(59)	(67)
Ca. Prostate II	400	310	220	145	110	—
(% Decrease)		(22)	(45)	(64)	(72)	
Percent decrease in PSA concentration corresponding to the value of first day						

Petterson *et al.* in 1995 (1) reported excellent immunochemical stability in vitro of free PSA from seminal fluid and of purified PSA. The immunoreactive forms of PSA in the peripheral circulation represent a much more complex setting than that of purified and stabilized proteins in buffer solutions. Currently the measurements of free PSA and total PSA are increasingly used to discriminate early focus of prostate cancer from benign conditions. It is very relevant to study both the long and short-term stability of the immuno-detectable PSA after vein puncture. Earlier studies (2) have shown that the concentration of PSA in serum may decrease considerably during storage at 4°C for 1-2 days. However, little or no changes were observed in the PSA-ACT fraction. The report by Leinone in 1993 (2) indicated that free PSA concentration was lost during storage at 4°C. Stenman *et al* in 1994 (3) reported that storage of serum more than 20 years at -20°C caused 40% mean loss of PSA immuno reactivity due to decrease of PSA-ACT complex. Both of these

studies indicated free PSA as well as PSA-ACT immuno reactivity loss during storage of serum sample.

Our results on the stability of total PSA and free PSA, in the sera frozen at -8°C to -10°C shown that both PSA and free PSA were stable for 4 months. This is similar to the observation made by Klaus Jung *et al.* in 1998 (4) in Germany. The difference is that they preserved the samples at -80°C and we preserved the sera at -10°C. BM multi-center international study has shown 37 days of stability at -20°C (5).

The present study at 2°C-8°C confirms that the reduction in the concentration of total and free PSA was progressive from 4th day onwards till 25th day of storage irrespective of the fact whether the samples were collected from healthy individuals or from patients of BPH or from cancer prostate. Compared to total PSA, the free PSA was less stable especially in samples with high values of PSA and free PSA. Our results were similar to the

Table 3. Stability of total and free prostrate specific antigen at -8 to -10°C

Days after storage	Total PSA (ng/ml)	Free PSA (ng/ml)
0	24.0	5.2
10	23.0	5.0
17	24.1	5.2
22	24.0	5.1
40	22.5	5.0
50	23.0	4.8
58	24.0	5.2
70	23.0	5.1
92	21.0	4.4
102	22.0	4.6
110	24.0	4.5
120	22.0	4.8
128	21.0	4.1
Coefficient variation	4.91	7.29

results of Klaus Jung *et al.* in 1998 (4). Samples for assessment of both free and total PSA could be safely refrigerated at 2°C to 4°C for less than 4 days. Boehringer Mannheim multicentre international study on Free PSA in 1996 (5) showed similarity with our results like the stability for 18 hours in most of their sera at 4°C. This is in contrary to the study Brett Simm *et al.* in 1991 (6), who showed that samples could be stored up to 14 days at 4°C.

Coagulated blood kept at room temperature for different duration of time has not shown any decline of both analytes. These findings were contrary to other studies that have shown slight decline in total and free PSA by delayed separation (7, 4).

In conclusion it was suggested that serum samples should be separated from the clot within five hours after vein puncture at room temperature. If the sample is collected at distant location from the investigating lab, the serum should be separated immediately and stored at -4°C as early as possible.

The analytes should be quantitated within 4 days if serum samples are refrigerated at 4°C. Samples could be stored at -10°C for 4 months, if the analytes are quantitated without any loss of immunoreactive fractions of free as well as total PSA.

REFERENCES

1. Pettersson, K., Piironen, T., Seppala, M., Liukkonen, L., Christensson, A., Matikainen, M.T., Suonpaa, M., Lovgren, T. and Lilja, H. (1995) Free and complexed prostate-specific antigen (PSA): *in vitro* stability, epitope map, and development of immunofluorometric assays for specific and sensitive detection of free PSA and PSA- α 1-antichymotrypsin complex. *Clin. Chem.* 41, 1480-1488.
2. Leinonen, J., Lovgren, T., Vornanen, T. and Stenman, U.H. (1993) Double-label time resolved immunofluorometric assay of prostate-specific antigen and of its complex with alpha-1-antichymotrypsin. *Clin. Chem.* 39, 2098-2103.
3. Stenman, U.H., Hakama, M., Knekt, P., Aromaa, A., Teppo, L. and Leinonen, J. (1994) Serum concentrations of prostate specific antigen and its complex with α 1-antichymotrypsin before diagnosis of prostate cancer. *Lancet.* 344, 1594-1598.
4. Jung, K., Philipp von Klinggraff, Brigitte Brux, Sinha, P., Schnorr, D. and Stefan, A.L. (1998) Preanalytical determinants of total and free prostate specific antigen and their ratio: Blood collection and storage conditions. *Clin. Chem.* 44, Nov.3, 685-688.
5. B.M. multicentre international study PSA free (1996) Boehringer Mannheim Lab Diagnostics, Mannheim, Germany.
6. Simm, B. and Gleeson, M. (1991) Storage conditions for serum for estimating prostate-specific antigen. *Clin. Chem.* 37 (1), 113-114.
7. Piironen, T., Pettersson, K., Suonapaa, M., Stenman, U.H., Oesterling, J.E., Lovgren, T. and Lilja, H. (1996) *In vitro* stability of free prostate-specific antigen (PSA) and prostate-specific antigen (PSA) complexed to α 1-antichymotrypsin in blood samples. *Urology.* 48 (6A), 81-87.