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The evaluation of markers of prostatic function

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Summary. The concentrations of three secretory proteins of the human prostate, including prostatic secretory protein of 94 amino acid residues (PSP94), prostate-specific antigen (PSA) and prostatic acid phosphatase (PAP), were measured by enzyme-linked immunosorbent assay (ELISA) in semen from a collective of patients suffering from various inflammatory diseases of the genital tract. In addition, levels of the conventional markers citrate, glucosidase and fructose were determined. As compared with semen from men exhibiting no inflammatory condition, only levels of glucosidase in cases of epididymitis and concentrations of PSP94 in the collective suffering from prostatitis showed significant reductions. The changes in the secretion of PSA, PAP, fructose and citrate in the semen of patients with inflammation of genital tract tissue were not significant at the 95% range of confidence.

Key words: Human prostate – Secretory proteins – Inflammation – Markers

The assessment of a range of biochemical markers in human semen is recommended to reveal disturbances of genital tract organs [7]. Among prostatic markers, the concentration of citrate and the activity of acid phosphatase are frequently used, as a good correlation exists with their concentrations in semen and since immunoassayable PAP also correlates well with its enzyme activity [27]. As demonstrated by Lilja and Abrahamsson [13], three predominant proteins are secreted by the human prostate gland: prostatic acid phosphatase (PAP) [16], prostate-specific antigen (PSA) [12, 22] and a 10.7-kDa protein termed the prostatic secretory protein of 94 amino acids (PSP94). The amino acid sequence of PSA has been reported by Watt et al. [23] and Schaller et al. [19]. The amino acid sequence of PSP94 was independently determined by Johansson et al. [8], Seidah et al. [20], and Akiyama et al. [2]. Recently, the cDNAs specific for these predominantly prostate-derived proteins were characterized and sequenced: PAP-specific cDNA [21], PSA-specific cDNA [14] and PSP94-specific cDNA [15]. The immunohistochemical distribution of the secretory proteins of the prostate in the parenchyma of prostate glands has been investigated by Abrahamsson et al. [1]. The localization of PSP94 in epithelial cells of the prostate has been demonstrated by in situ hybridization using PSP94-specific cDNA as well as by immunohistochemistry [3]. The present study compares the prostatic secretion of these proteins with that of citrate, an established marker of prostatic function; fructuse, an indicator of seminal vesicle secretion; and glucosidase, a marker of epididymal function [7].

Patients and methods

Semen

Semen was obtained by masturbation from men attending the Outpatient Department for Prostatitis and Urological Andrology, Giessen. Symptomatic patients selected for the study exhibited acute epididymitis (collective E), chronic prostatitis (collective P) or signs suggesting male accessory-gland infection (collective A) without a defined localization of the infectious focus.

Acute epididymitis. Men with acute epididymitis suffered from a swollen and tender epididymis during palpation, with an acute infiltration of the organ being the main symptom [28]. All patients underwent a standardized examination procedure, including gram staining and light microscopy of a urethral swab; the same specimen was used for subsequent culture for *Neisseria gonorrhoeae* and *Chlamydia trachomatis*. Then, initial and midstream urine samples were obtained and examined microscopically as well as by a routine bacteriological procedure [9]. Patients were initially treated with 100 mg doxycycline twice a day for at least 2 weeks and were reexamined by the same procedure after 3 days. At this reevaluation date, semen was obtained for further analysis. The microbiological classification of acute epididymitis was not a subject of the present study and was not further considered.

Chronic prostatitis. Patients with chronic prostatitis had shown a medical history of symptomatic prostatitis for at least 1 year. For diagnosis, all men underwent a standardized investigation program based on the "four-specimen-technique", including quantitative

Table 1. Matrix of correlation coefficients of various parameters for 32 samples of normal semen

	PSP94	PAP	PSA	Glucosidase	Fructose	Citrate
PSP94 (mg/ejaculate)	1	0.686[a]	0.696[a]	0.225	0.048	0.823[a]
PAP (mg/ejaculate)	0.686[a]	1	0.447	0	0.049	0.853[a]
PSA (mg/ejaculate)	0.696[a]	0.447	1	0.259	0.352	0.642[a]
Glucosidase (mIU/ejaculate)	0.225	0	0.259	1	0.408	0.214
Fructose (μmol/ejaculate)	0.048	0.049	0.352	0.408	1	0.084
Citrate (μmol/ejaculate)	0.823[a]	0.853[a]	0.642[a]	0.214	0.048	1

[a] Correlation coefficients of ≥ 0.6

determination of common bacteria [17]. Material obtained on urethral swabs after prostatic massage was cultivated for *C. trachomatis* [4]. Leucocyte analysis of prostatic secretions was performed in a fresh smear of expressed prostatic secretions (EPS, 1,000× as well as in 3 ml cytocentrifuged urine specimens obtained before and after prostatic massage [24]. Numbers of leucocytes were considered to be significant when $\geq 10 \times 10^3$ leucocytes in a smear of EPS and/or $\geq 4 \times 10^3$ granulocytes in the sediment of centrifuged urine after prostatic massage (UB3) were detected in specimens of leucocyte-free midstream urine [11, 17, 26]. A classification of prostatitis was based on duplicate investigation; the hallmark of this diagnosis was evidence of increased numbers of leucocytes in prostatic secretions. In case of leucocyte-free prostatic secretions, patients exhibiting symptoms of prostatitis were classified as having prostatodynia and were excluded from analysis. Subjects suffering from prostatitis who displayed increased numbers of leucocytes were diagnosed as having chronic bacterial prostatitis when a typical pattern of gram-negative bacteria or enterococci, was observed, i.e. $<10^3$ colony-forming units (cfu)/ml in first-voided urine (VB1) and bladder urine (UB2), $>10^4$ cfu/ml in EPS and $>10^3$ cfu/ml in UB3, but at least a 10-fold increase in the number of microorganisms in UB3 as compared with VB1 [17]. Evidence of high numbers of ureaplasmas (ureaplasma-associated) or positive urethral findings of *C. trachomatis* were not considered in this study. For ejaculate analysis, semen specimens were obtained from all patients within <7 days after the last prostatic massage (including the above-mentioned analysis), i.e., all men were instructed to abstain from sex for at least 3 days and for a maximum of 6 days.

Accessory-gland infection. Patients were classified as having accessory-gland infection without a proven infectious focus according to a suggestion published by Comhaire et al. [5]. The hallmark of classification was evidence of $\geq 10^6$ peroxidase-positive leucocytes in the ejaculate [29]. Clinically, acute epididymitis and acute or chronic urethritis (discharge or increased leucocyte numbers, in the initial urine) were excluded. Urethral swabbing for *C. trachomatis* and *N. gonorrhoeae* was negative in every case [25]. The four-specimen technique did not give any hint of bacterial infection; furthermore, increased numbers of leucocytes could not be demonstrated in prostatic secretions, thus excluding a diagnosis of prostatitis at the time of examination [11, 17]. Nevertheless, all of these men reported a typical medical history of recurrent urogenital infections and a history of prostatitis without a defined localization of infection.

Control group

Semen samples from asymptomatic men who had recently the clinic because of a barren marriage served as controls (collective N). This group comprised normozoospermic samples ($>20 \times 10^6$ spermatozoa/ml semen) from patients whose partners were women with tubal sterility who were attending a gynecology clinic. These men showed no obvious signs of inflammation, i.e. $<10^6$ peroxidase-positive leucocytes/ml, no increase in the number of leucocytes in prostatic secretions, no clinical signs of urethritis or epididymitis and negative urethral swabbing for *C. trachomatis* as well as *N. gonorrhoeae.* After liquefaction, semen was centrifuged at 2,000 *g* for 10 min and then frozen at −70°C for 3–4 years. Samples were thawed and mixed well between assays.

Assays

Concentrations of PSA and PAP were measured by commercial ELISA (Hybritech, CIS) and levels of citrate, fructose and α-glucosidase (total enzymic activity at neutral pH) were determined by spectrophotometric methods described elsewhere [6, 7]. PSP94 was determined by a recently described ELISA [10]. All determinations for PAP, PSA and PSP94 were carried out in duplicate. The intra-assay variation in ELISA measurements was $\leq 8\%$. The output of each marker per organ was obtained by multiplying its concentration in semen times its volume.

Statistical analysis

Because the data obtained from the respective semen collectives did not follow a standard distribution, statistically significant differences between groups were detected by calculating the median value at different confidence levels [18]. Confidence ranges of median values for disease groups that did not overlap with those of the normal collective were considered to be significantly different. Calculations were performed using the program StatView on a Macintosh Plus computer.

Results

Using the experimental data measured in the normal non-inflammatory semen samples (group N) as a reference, a correlation of the parameters was attempted. The results depicted in Table 1 clearly indicate a significantly positive correlation among the protein markers PAP, PSA and PSP94 as well as between these markers and citrate; inflammation of genital tract tissue did not affect this correlation. No correlation with fructose or glucosidase was detected.

In a first attempt to illustrate differences among the parameter values of the semen collectives, the percentile plots of the data were inspected (Fig. 1). A clear-cut picture was derived from a comparison of the percentile plots of the fructose and PSA levels measured in groups N, A, E and P; the median value as well as the 90th percentile distribution of values apparently showed no significant difference. In the percentile plot, glucosidase appeared to be uniquely related to an inflammatory condition of the epididymis: almost 75% of all glucosidase values obtained

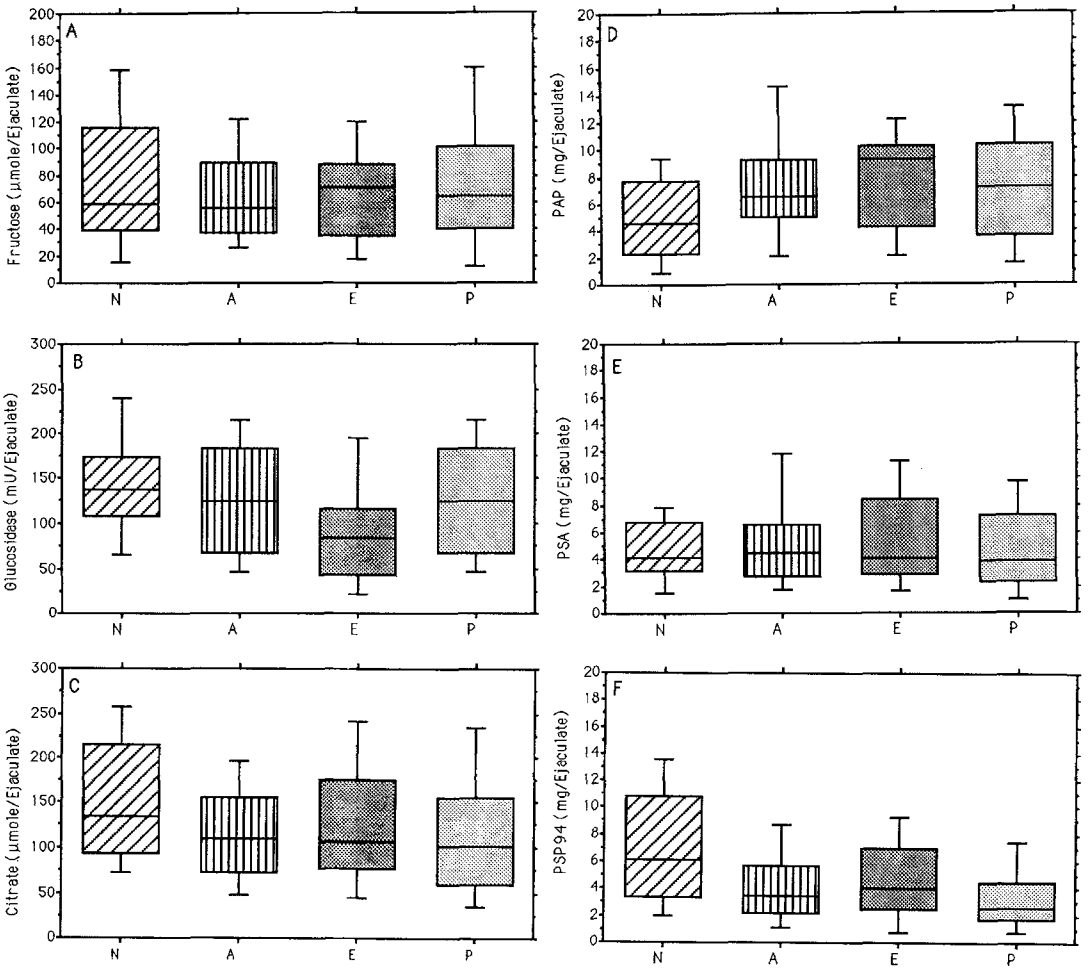

Fig. 1. Schematic representation of percentiles of semen parameters. Abbreviation of semen collectives: *N*, normozoospermic, non-inflammatory; *A*, adnexitis; *E*, acute epididymitis; *P*, chronic prostatitis. The lines of individual boxes represent, from top to botton, the 90th, 75th, 50th, 25th and 10th percentiles

for group E were found to be lower than the 25th percentile of the normal values. In contrast, the distribution of glucosidase values for groups A and P were similar to those of the normal collective.

A perplexing property of PAP secretion by individuals suffering from inflammatory processes was indicated by the percentile plots for groups A, E and P. The median values for these groups were apparently elevated as compared with those obtained for the normal group. However, the distribution of values between the 25th and the 75th percentile was rather broad and overlapped with that observed for group N.

A close inspection of the distribution of citrate values for group P in the percentile plot disclosed that >25% of these values were lower than the lowest citrate levels measured in group N. On the other hand, the median levels of citrate found in all groups did not differ significantly.

The percentile plot of PSP94 concentrations illustrates differences in the values found for groups A and P relative to the normal collective. In the case of group A, 75% of all values were below the median normal value, and the distribution of values between the 25th and the 75th percentile was narrow. Moreover, in group P the median level was well below the 25th percentile of the values for group N.

To evaluate the statistical significance of the differences displayed in the percentile plot, we calculated confidence ranges for the median levels of all parameters in the different groups. The surprising result (see Table 2) was that the citrate values found for group P as compared with group N were statistically insignificant, even at the lower confidence range of 95%. However, at both the 95% and the 99% confidence range, the parameters glucosidase and PSP94 appeared to be significantly correlated with epididymitis and prostatitis, respectively. Various ratios of marker concentration were calculated to determine whether better discrimination among the groups with different inflammatory diseases could be achieved, but improved differentiation was not obtained.

Discussion

Measurement of a range of markers in semen as indices of accessory-gland function provides additional data that can help to confirm a clinical diagnosis [7]. The inflamma-

Table 2. Amounts of fructose, citrate, glucosidase and prostatic secretory proteins in seminal plasma in various inflammatory conditions

Parameter	Diagnosis	*n*	Range	Median (95% confidence range)	Median (99% confidence range)
Fructose (µml/ejaculate)	N	32	2 -273	40 ≤ 59.7 ≤ 98	38.5 ≤ 59.7 ≤ 111.6
	A	39	6 -184	39.6 ≤ 56.1 ≤ 76.4	39 ≤ 56.1 ≤ 79.75
	E	25	11 -122	39.6 ≤ 70.8 ≤ 80.4	33.6 ≤ 70.8 ≤ 87.6
	P	62	4 -241	55.2 ≤ 64 ≤ 72.3	46.2 ≤ 64 ≤ 73
Citrate (µmol/ejaculate)	N	32	69 -476.7	98 ≤ 133.8 ≤ 178.5	93 ≤ 133.8 ≤ 206
	A	38	32 -371	76.8 ≤ 110.6 ≤ 146.4	72.5 ≤ 110.6 ≤ 151.2
	E	23	32.4 -244.2	86.4 ≤ 106 ≤ 144	69.6 ≤ 106 ≤ 183
	P	62	11.4 -360	69.6 ≤ 102.05 ≤ 125.5	64.2 ≤ 102.05 ≤ 131.6
Glucosidase (mIU/ejaculate)	N	32	42 -299.5	126.6 ≤ 138.75 ≤ 166.2	115.5 ≤ 138.75 ≤ 171
	A	42	5.8 -308.4	91.8 ≤ 114.05 ≤ 147.4	88.5 ≤ 114.05 ≤ 158.5
	E	26	11.7 -333.6	42 ≤ 84 ≤ 113.4[a]	41 ≤ 84 ≤ 116.8[a]
	P	43	11.4 -374.1	101.75 ≤ 124.2 ≤ 146	86.5 ≤ 124.2 ≤ 165.6
PAP (mg/ejaculate)	N	31	0.28- 27.49	2.948 ≤ 4.58 ≤ 6.465	1.998 ≤ 4.58 ≤ 7.08
	A	40	0.32- 16.05	5.16 ≤ 6.525 ≤ 8.25	5.06 ≤ 6.525 ≤ 8.42
	E	22	0.44- 13.0	4.2 ≤ 9.295 ≤ 10.12	2.94 ≤ 9.295 ≤ 10.32
	P	62	0.44- 21.51	4.86 ≤ 7.2 ≤ 8.55	4.47 ≤ 7.2 ≤ 8.82
PSA (mg/ejaculate)	N	32	0.16- 10.98	3.35 ≤ 4.175 ≤ 5.76	3.12 ≤ 4.175 ≤ 6.45
	A	39	0.66- 16.6	3 ≤ 4.1 ≤ 5.28	2.75 ≤ 4.1 ≤ 5.88
	E	23	1.26- 16.88	3.32 ≤ 4.05 ≤ 7	2.68 ≤ 4.05 ≤ 8.72
	P	61	0.28- 20.25	2.59 ≤ 3.875 ≤ 5.43	2.34 ≤ 3.875 ≤ 6.04
PSP94 (mg/ejaculate)	N	32	1.16- 20.3	4.12 ≤ 6.07 ≤ 9	3.63 ≤ 6.07 ≤ 9.18
	A	41	0.54- 16.2	2.648 ≤ 3.452 ≤ 4.475	2.252 ≤ 3.452 ≤ 4.794
	E	24	0.44- 13.38	2.62 ≤ 3.998 ≤ 6.81	1.892 ≤ 3.998 ≤ 6.99
	P	61	0.32- 17.57	1.864 ≤ 2.574 ≤ 3.064[a]	1.832 ≤ 2.574 ≤ 3.108[a]

[a] Confidence ranges of median values that show only insignificant, if any, overlap with those of the normal group

tory conditions described herein did not appear to influence the seminal vesicles, since fructose secretion in the disease groups did not differ from that in the controls. As expected, total glucosidase activity was significantly lower in patients with epididymitis than in the control group. Although the secretion of the accepted prostatic marker citrate was apparently reduced not only in patients with prostatitis as compared with controls, as anticipated, but also in those with epididymitis or adnexitis, as previously demonstrated [7], the differences did not reach statistical significance.

Of the new markers examined in the present study, only PSP94 exhibited a reduced secretion in cases of prostatitis. Of interest is the observation that the secretion of PSA was not influenced by the inflammatory condition. Clearly, different mechanisms for the synthesis and secretion of the prostatic proteins are evident.

The opposing trends in the secretion of PAP and citrate as compared with those in the normal group requires comment. Usually, this enzyme activity is well correlated with the ejaculate content of citrate [27]. In the present study, citrate secretion was reduced and PAP secretion was increased relative to the values found for the control group. If corresponding individual data pairs are compared, the known correlation between the secretion of citrate and that of PAP remains valid for the semen samples from patients exhibiting inflammation of the genital tract. As PSP94 secretion was also decreased in patients with adnexitis and epididymitis, the secretion of this protein would appear to be more sensitive than citrate to inflammation. We therefore advocate measurement of PSP94 levels in human semen as a useful marker of prostatic function.

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