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Elastase: A Predictive Marker of Inflammation and/or Infection

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The present study was undertaken to estimate elastase in biological fluids and assess its usefulness as an indicator of inflammation/infection. Elastase was measured in seminal plasma, serum, urine, and cervical specimens using a specific substrate and was expressed in arbitrary units (AU). It was found to be stable over a period of 3 weeks. The intra- and inter-assay variation of elastase assay was between 2.3 to 6.8% and 7.3 to 9.9%, respectively. The assay was validated by comparing it with other methods that are available for the detection of infections. Sensitivity of the assay indicating inflammation/infection in these samples varied between 70.9 to 87.3%. The results obtained suggest that the presence of elastase in specimens may be used as a nonspecific indicator and could be used to screen inflammation/infection in a limited resource setting. J. Clin. Lab. Anal. 18:153–158, 2004. © 2004 Wiley-Liss, Inc.

Key words: elastase; inflammation; infection

INTRODUCTION

As part of the inflammatory response, infection triggers the release of various enzymes from polymorphonuclear leukocytes. One of the important events during this process is the discharge of large amounts of proteases such as elastase, cathespin G, and collagenase. Elastase is released from the azurophilic granules of polymorphonuclear leukocytes during phagocytosis, degranulation, or cell death. Extracellular release is considered an important inflammatory mediator, and is therefore considered to be a suitable indicator of inflammation (1,2). Blood, urine, cervical, and semen specimens are used, in general practice, to detect infections especially sexually transmitted infections (STI). Previous reports suggest that the estimation of elastase levels in seminal plasma enables a reliable and rapid diagnosis of silent male genital tract inflammation/infection (3). However, the acceptability of obtaining semen specimens in a clinical setting has been a major obstacle, in contrast to the ease of obtaining urine for testing STIs. Similarly, blood specimens are routinely taken for the diagnosis of sexually transmitted infections, e.g., human immunodeficiency virus (HIV), hepatitis B (HBsAg), and syphilis, while cervical specimens are routinely used for diagnosis of lower genital tract infections in women. The antimicrobial activity of cervical mucus is regarded as the local defense mechanism against ascending infections by the vaginal bacterial flora. Furthermore, fluctuations in elastase levels have been observed in cervical mucus in obstetric and gynecologic infection (4).

In the present study, we evaluate the presence of elastase in different biological specimens and assess its use as an indicator of inflammation/infection.

MATERIALS AND METHODS

Study Specimens

Semen, urine, blood, and cervical swabs were collected for the detection of elastase.

Semen

A total of 395 semen specimens were collected from the male partners of infertile couples with no clinical signs of genital tract infection. Their ages ranged from 22 to 56 years. Semen samples were collected after

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4.16 ± 2.7 days of sexual abstinence and processed for routine analysis (5). This included microscopic analysis for detection of amorphous particulate, pus cells, sperm count, agglutination, and viscosity by needle-syringe method (6). Semen specimens were centrifuged at 3,000 rpm for 5 min to separate seminal plasma and then used for elastase estimations.

Serum

Elastase was estimated in serum from the blood specimens taken for first-time diagnosis of HIV (n = 337) by ELISA (Genedia HIV 1/2 ELISA 3.0; Greencrose Life Science Corp., Yongin-Shikyunnggi-do, Korea and Comb AIDs; Span Diagnostics Ltd., India), HBsAg (n = 77) by ELISA (Hepanostika; Organon Teknika, Boxtel, The Netherlands), and syphilis by VDRL (n = 56) in different dilution (Tulip Diagnostics Pvt. Ltd., Surat, India) at the Microbiology Department of Seth G.S. Medical College, Parel, Mumbai, India. The age range of subjects included was 1 month to 53 years (mean ± SD: 29.9 ± 10.9). These cases had clinical symptoms such as a history of loss of appetite and weight loss, or they had an infected spouse or parents.

Urine

First-voided morning urine specimens (n = 127) were collected from males (ages 18 to 45, mean ± SD: 30.3 ± 8.3 years) attending the Skin and Venereal Diseases OPD of the Seth G.S. Medical College, Parel, Mumbai, India. An aliquot of 2 mL was used to estimate catalase using the Uriscreen kit (Diatech Diagnostics Ltd., Nessziona, Israel) according to the manufacturers' instructions for detection of urinary tract infections (7). Another aliquot was processed for elastase assay and 50 mL midstream urine was centrifuged to collect the sediment for microscopic examination. Each individual was examined for clinical signs of any genital infection.

Cervical Specimens

Both vaginal and cervical specimens were collected from women (n = 303) between the ages of 18 to 45 years (mean ± SD: 28.69 ± 6.79 years), attending the Gynecology OPD of K.E.M. Hospital, Mumbai, India. The vaginal smears were processed for wet mount to detect *Candida albican*, *Trichomonas vaginalis*, and gram stain for bacterial vaginosis (BV), respectively. BV was evaluated using the Nugent score (8). Cervical specimens were dissolved in 1 mL normal saline and used for elastase assay as well as for PCR diagnosis of *Chlamydia trachomatis* (9). An examination of the cervix with a speculum in order to record clinical signs of infection/inflammation was also evaluated.

Control Specimens

Specimens obtained from healthy volunteers were analyzed for routine semen parameters; Erythrocyte Sedimentation Rate (ESR), White Blood Cell Count (WBC), and Differential Count (DC) were done in each blood specimen; routine microscopic and catalase assay were done in urine; wet smear, pap, and gram stain were done in vaginal specimen. Specimens that were considered normal on the basis of these tests were included as controls. These included: 1) 10 semen specimens from proven fertile males (mean age ± SD: 34 ± 4.5 years); 2) 13 blood specimens from both males and females (mean age ± SD: 23.1 ± 3.1 years); 3) 14 urine specimens from both males and females (mean age ± SD: 19.9 ± 9.0 years); and 4) 9 cervical specimens from women (mean age ± SD: 26.0 ± 5.0 years) who were coming to Gynecology OPD for family planning advice.

Elastase Assay

The elastase assay was carried out using a specific substrate, L-pyroglutamyl-L-prolyl-L-valine-p-nitroanilide, dissolved in DMSO (25 mg in 5.6 mL of DMSO) as described in Kramps et al. (10) and Fraser et al. (11). In brief, 50 µl of each specimen in duplicate was mixed with 100 µl of 0.1 M Tris HCl-NaCl buffer (pH: 8.3, 0.96 M NaCl) in microtitre plates and kept at 37°C for 10 min. A freshly prepared 50 µl of the 1:5 diluted substrate solution (2 mM) was added to the buffered specimen and the absorbance or optical density (OD) was immediately taken at 405 nm. A substrate blank reading was also recorded simultaneously. The plates were then incubated at 37°C for 3 hr in the dark. At the 3rd hr, the OD at 405 nm was again recorded against the substrate blank. The change in the OD over the 3 hr period was calculated for each sample and the result was expressed in arbitrary units (AU). The mean value of each sample was taken into consideration for expression of elastase in AUs.

Stability in Different Specimens

To determine the degradation of elastase between the time of specimen collection and analysis, the elastase values were determined in three each of the four types of biological specimens. This included one specimen from the control and two from the study specimens. This was carried out during three consecutive weeks.

Precision

There were seven assays for a single specimen, and three such specimens were analyzed from each category, i.e., from semen, serum, urine, and cervical specimens, to find out the intraassay coefficient of variation. Similarly, three samples from each category were tested on seven consecutive days to determine the interassay coefficient of variation.

RESULTS AND DISCUSSION

Cut-Off Values

The normal values (range), mean, SD, and the cut-off value (mean + 2 SD) of elastase for different specimens are expressed in AUs in Table 1. Specimens whose elastase values were more than the respective cut-off value were considered as positive and the rest were accepted as negative.

TABLE 1. Normal elastase values as expressed in arbitrary units (AU) in different biological samples

	Semen	Serum	Urine	Cervical swab
N	10	13	14	9
Range	0.05–0.433	0.0–0.077	0.0–0.037	0.0–0.066
Mean	0.303	0.020	0.004	0.031
SD	0.120	0.027	0.010	0.020
Cut off value (Mean + 2 SD)	0.543	0.074	0.024	0.071

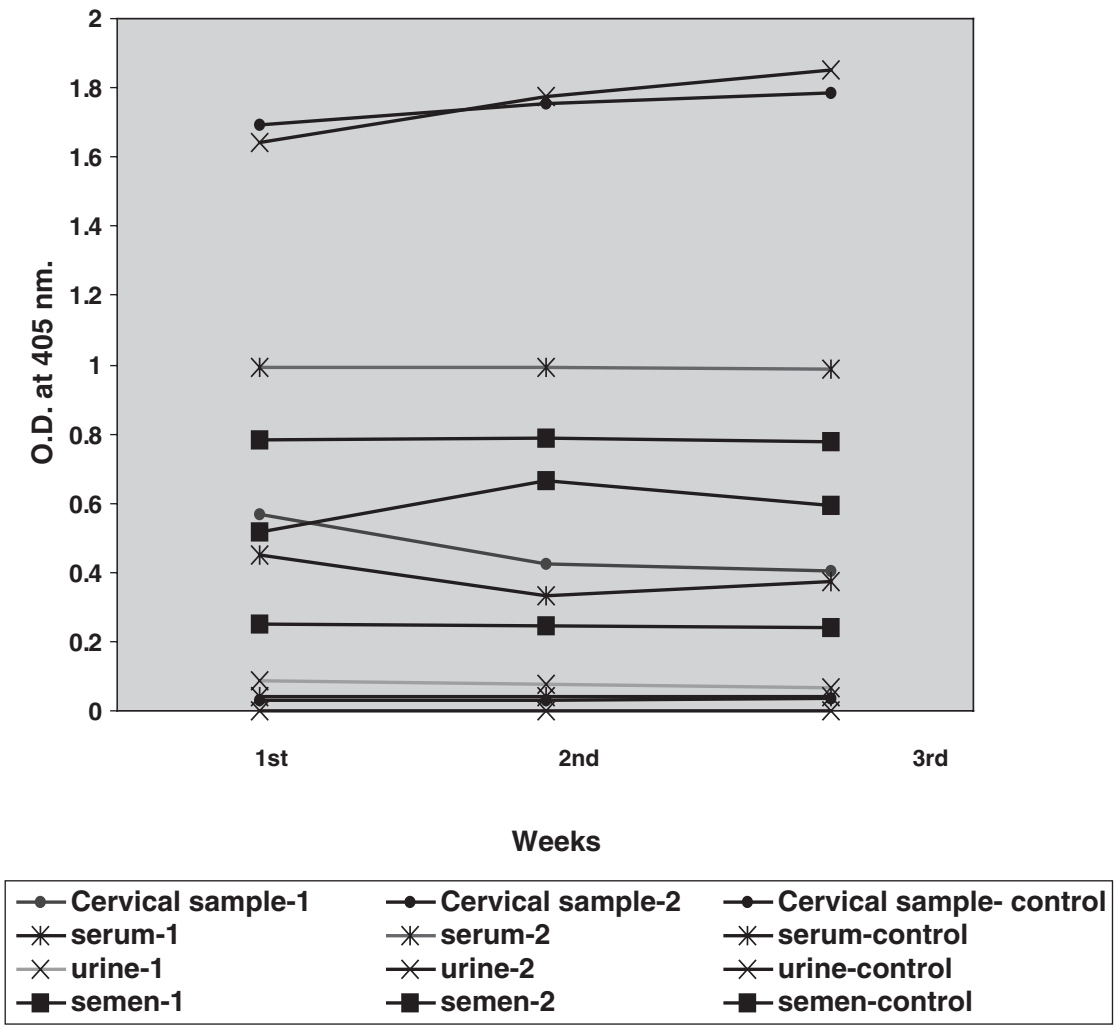

Fig. 1. Stability of elastase level in different biological samples over a period of time.

Stability in Different Specimens

As reflected in their OD, no significant change was observed in elastase values of different types of biological specimens (Fig. 1). The enzyme was found to be stable over a reasonable period of time, 3 weeks as observed in the present study. Thus, specimens can be transferred to diagnostic centers without loss of enzyme activity.

Precision

The intra- and interassay variations for each type of specimen was within the limit, i.e., the intraassay variation was 2.3, 2.6, 6.8, and 3.4%, respectively, for semen, serum, urine, and cervical samples, while the interassay variation of these samples was 7.3, 9.9, 9.6, and 8.4%, respectively.

TABLE 2A. Presence of elastase in semen (n = 303) and its comparison with routine semen analysis (RSA) to detect inflammation/infection

RSA	Amorphous particles	Pus cells	Viscosity	More than one abnormalities	Total samples infected	Normal samples
	(a)	(b)	(c)	(d)	(e) (a + b + c + d)	(h)
Elastase	+ ve	+ ve	+ ve	+ ve	+ ve	− ve
+ ve	73	58	12	67	210	1
− ve	18	15	6	13	52	40
Total	91	73	18	80	262	41

Assay sensitivity, 80.2%; assay specificity, 97.6%; assay accuracy, 82.5%.

TABLE 2B. Presence of elastase in serum and its comparison with detected infection

Infection	HIV n = 337		VDRL n = 56		HBsAg n = 77		Total	
Elastase	+ ve	− ve	+ ve	− ve	+ ve	− ve	+ ve	− ve
+ ve	204	5	30	2	44	1	278	8
Treated and − ve	60	–	6	–	8	–	74	–
− ve	49	19	5	13	11	13	65	45
Total	313	24	41	15	63	14	417	53

Assay sensitivity, 81.0%; assay specificity, 84.9%; assay accuracy, 81.6%.

TABLE 2C. Presence of elastase in urine and its comparison with other methods of detection of infection

Other method	Catalase assay		Microscopic analysis		Clinical history, signs and symptoms		Total	
Elastase	+ ve	− ve	+ ve	− ve	+ ve	− ve	+ ve	− ve
+ ve	42	0	47	1	48	0	48	1
− ve	9	76	8	71	62	17	7	71
Total	51	76	55	72	110	17	55	72

Assay sensitivity, 87.3%; assay specificity, 98.6%; assay accuracy, 93.7%.

TABLE 2D. Presence of elastase in cervical samples and its comparison with other method for detection of inflammation/infection

Infection	Clinical signs and symptoms						Total	
Elastase	+ ve	− ve	*C. trachomatis*	Trichomonas, Candida	BV	Other non-specific infection	+ ve	− ve
+ ve	132	22	29	6	31	50	132	22
− ve	99	50	14	0	10	6	54	95
Total	231	72	43	6	41	56	186	117

Assay sensitivity, 70.9%; assay specificity, 81.2%; assay accuracy, 74.9%.

Elastase in Different Biological Fluids

For each type of specimen, the number of specimens found to be positive and negative for the presence of elastase were determined and compared with other methods for the detection of infection (Table 2A–D).

In Semens

Microscope analysis detected infection in 262 (66.33%) of the 395 semen specimens on the basis of one or more abnormal parameters, such as the presence of amorphous particulates ($>++$/high power field [hpf]), pus cells (>5/hpf), or were hyperviscous, and 210 (80.2%) of them had high units of elastase (Table 2A). The rest of the samples ($n=52$) that were negative for elastase but were positive in routine microscopic analysis might have been contaminated post ejaculation leading to a false positive result (3). The other 92 specimens had some other abnormalities such as low sperm count, volume, motility, or agglutination, which were not taken into consideration. It is well established that silent male genital tract infection can only be diagnosed by laboratory parameters. Even in combination, however, these factors gave no proof for silent male genital tract inflammation/infection. Moreover, as a single parameter, they are inadmissible for a correct diagnosis (12). Therefore, with a sensitivity of 80.2%, elastase assay can quickly predict if any inflammation/infection exists.

In Serum

Of the 470 serum specimens, 417 were positive for HIV, HBsAg, or VDRL (titre $\geq$ 1: 64), and elastase was detected in 278 (66.7%) (Table 2B). The other 139 infected cases had normal elastase values. However, the clinical history of 74 of the subjects suggested chronic rather than acute infection, and they had already been on treatment. Elastase results of these 74 were excluded, and only 343 infected specimens were taken for correlation. The sensitivity was observed to be 81.0%. Fifty-three specimens collected from clinically symptomatic subjects were declared to be free of HIV, HBsAg, and syphilis, and eight (15.1%) of them had high elastase units indicating the presence of a probable underlying inflammation. Screening methods for these infections are not always accessible in a resource-poor setting, where a measurement of elastase in the serum of suspected cases may provide a quick nonspecific screening for infection.

In Urine

Microscopic examination of 127 urine specimens showed presence of pus cells, epithelial cells, or both (>5/hpf) in 55 specimens. Clinical signs and symptoms including urethritis, urethral discharge, pruritis, burning, pain on micturation, genital itching, presence of warts, ulcers, and a history of STI-exposures were identified in 110 cases, and 51 of them were positive for urine catalase. Urine specimens were considered infected if positive by routine microscopy, catalase assay, and the subjects had signs of infection. This criteria identified 55 (43%) samples as having infection and 48 (87.3%) of them had high units of elastase (Table 2C). These findings suggest that the elastase assay has a sensitivity of 87.3% in assessing inflammation/infection, and thus its use is supported in screening programs. This was also reported earlier in Fraser et al. (11).

In Cervical Specimen

Among the 303 cervical specimens, 231 were from subjects with cervicitis, vaginitis, or they had a bad obstetric history or had documented infertility. Microscopic analysis, both wet mount and gram stain, revealed infections such as *Trichomonas vaginalis*, Candida, BV, Moniliasis, and *Chlamydia trachomatis* (by PCR). Nonspecific infections were identified by the presence of pus cells (>5/hpf), vaginal pH >4.5 in 56 specimens. In total, infection was identified in 186 (61.4%) when clinical signs or symptoms, microscopic and other analysis, were taken together and 132 (70.9%) of them had high elastase (Table 2D). In the rest, the presence of BV or other infections might not have induced inflammation and hence there was no raise in elastase.

These observations showed that the sensitivity of this test for screening inflammation in different biological specimens only varied from 70.9 to 87.3%. Infection, both acute and chronic, may not always lead to inflammation. Self-treatment and treatment with antibiotics can lead to a significant decline in elastase concentration, most probably due to the elimination of infective agents and the additional antiinflammatory effect of drugs. All these might have influenced the sensitivity and specificity of the assay. Similarly, the sensitivity and specificity was only 52.8% and 75%, respectively, when correlated with the clinical findings of subjects from whom urine and cervical specimens were collected. Hence, in a resource-poor setting solely reliant on syndromic treatment, a general indication of inflammation, i.e., the presence or absence of elastase, could be of diagnostic importance. It has the potential for clinical application in testing STIs among males using their urine samples and thus may help in removing the major obstacle of testing for STIs. Finally, we suggest that assessing elastase in different biological

specimens could quickly screen any inflammation/infection.

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