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Prolactin and α-1, 4-glucosidase activity in normal and poorly coagulated human semen

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Summary
Prolactin and α-1,4-glucosidase levels in seminal plasma were measured in poorly coagulated (I), deficiently coagulated (II) and normally coagulated (III and IV) human ejaculates having 0–20%, 21–50% and 51–100% coagulum respectively 4 min after emission. The prolactin concentration (ng ml^{-1}, mean ± SEM) in poorly coagulated (5.2 ± 0.48) and deficiently coagulated (7.6 ± 0.72) samples was significantly lower than in the normally coagulated groups III (51–75% coagulum, 8.2 ± 0.43) and IV (76–100% coagulum, 9.9 ± 0.59) as well as the presumably fertile samples (9.2 ± 0.74). A highly significant positive correlation was observed between the prolactin level and the percentage coagulum of the ejaculates (r = 0.686, n = 58, $P<0.001$). In contrast, the epididymal marker, α-glucosidase showed no relationship to seminal coagulation.

Keywords: α-1,4-glucosidase, coagulation, prolactin, semen.

Introduction

Human seminal plasma contains exogenous, biologically active and immunoactive prolactin (Dattatreyamurty & Sheth, 1977). It is well known that this pituitary hormone has a direct effect on the growth and secretory activity of male accessory organs (Ensor, 1978), and specific binding sites for prolactin exist in several male accessory glands as well as in the testis (Aragona & Friesen, 1975; Barkey *et al.*, 1979). Prolactin acts on the accessory glands both independently and in synergy with testosterone by promoting its synthesis by increasing the number of luteinizing hormone receptors on Leydig cells (Zipf *et al.*, 1978; Mann & Lutwak-Mann, 1981). This peptide hormone also influences the uptake of testosterone, dihydrotestosterone and zinc, the synthesis of protein and the secretion of citric acid by the accessory organs (Grayhack & Lebowitz, 1967; Resnick *et al.*, 1974; Johansson, 1976; Slaunwhite & Sharma, 1977). The prolactin level in human seminal plasma may therefore be related to the associated accessory-gland activity.

Freshly ejaculated human semen is a heterogeneous mixture of the secretions from three organs of the male genital tract. Normally 50–80% of the total ejaculate volume is contributed by the seminal vesicles about 15–40% by the prostate and

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3–5% by the testes and epididymides (Tauber *et al.*, 1980; Zaneveld & Tauber, 1981; Wetterauer, 1986). However, in 20–25% of infertile couples the semen either fails to coagulate or shows only up to 25% coagulation despite semen volume remaining within the normal range of 2–6 ml (Mandal & Bhattacharyya, 1987c; 1990a; Mandal *et al.*, 1989). These poorly coagulated ejaculates exhibit decreased seminal vesicular activity (Mandal & Bhattacharyya, 1985a, c, 1986b, 1987b, 1990a). Seminal components, which are predominantly secreted by the seminal vesicles, such as protein, fructose, *N*-acetylamino sugars, phosphate, choline, sialic acid and amino sugars are significantly lower in the poorly coagulated samples than in ejaculates showing normal coagulation. Deficiency in coagulum formation is also associated with lower osmotic pressure, buffering capacity and poor maintenance of sperm motility (Mandal & Bhattacharyya, 1988a). Hitherto, the prolactin contents of human seminal plasma has not been examined with respect to coagulation, a characteristic that largely influences the seminal plasma constituents (Mandal & Bhattacharyya, 1985b, 1987a, 1988b, 1990b).

The objective of the present investigation was to study the relationship between the percentage coagulum of human ejaculates and their seminal plasma prolactin level and α-1, 4-glucosidase activity, the latter deriving mainly from the epididymis (Tremblay *et al.*, 1982; Casano *et al.*, 1987) under androgen control (Gunaga *et al.*, 1971).

Materials and methods

Semen samples

Semen samples were obtained by masturbation from four vasectomized men and 58 infertile couples (age of men 25–45 years) attending the University Hospital of Calcutta after a requested abstinence period of 6.5 (mean ± SEM = 6.7 ± 0.1) days. Donors had no history of impotence, cryptorchidism, mumps, orchitis, varicocele, venereal disease, gynaecomastia or galactorrhea. None took any medicine to improve their fertility potential. No sample with an ejaculate volume outside the range of 2–6 ml or with a white cell count (Nahoum & Cardozo, 1980) above 3×10^6 ml^{-1} was included in this study. All samples liquefied completely within 22 min of ejaculation at room temperature. The sperm count and the percentage motile sperm were determined within 2 h of emission using a Makler Counting Chamber, as described earlier (Mandal *et al.*, 1989). Prostatic fluid was obtained from a man aged 28 years with congenital absence of the vasa deferentia and seminal vesicles (Molnar *et al.*, 1971). This ejaculate had a liquid consistency, a volume of 0.6 ml, a pH 6.0, no sperm, 0.19 mg ml^{-1} fructose and 770 μg ml^{-1} zinc. After liquefaction, seminal plasma was obtained by centrifugation at 4°C for 30 min at 10,000 ***g*** and stored at −70°C until used.

Determination of the percentage coagulum

The amount of coagulum in ejaculates was determined 4 min after emission as it was observed that significant liquefaction of the coagulum occurred after 4 min (Mandal & Bhattacharyya, 1987b). The widely varying coagulum content of the fresh ejaculates was determined using nylon bags with a 37-μm mesh size that

separates the gel-like coagulum from the liquefied part (Mandal & Bhattacharyya, 1985b). This mesh size was used to measure the liquefaction time of normally coagulating, poorly coagulating and slowly liquefying human ejaculates (Mandal & Bhattacharyya, 1986a, 1988b, 1990a). Ejaculates were received within 5 s of emission and were allowed to liquefy at room temperature. Immediately after collection, the whole ejaculate was placed into the nylon bag, keeping it inside a graduated vial. To follow the rate of liquefaction, the bag was lifted above the liquefied part every 2 min and the amount of fluid in the container recorded until the completion of liquefaction. The amount of coagulum 4 min after ejaculation was obtained by subtracting the liquefied volume at that time from the total volume, and the resultant value was expressed as a percentage of the total. Details of this method have been presented elsewhere (Mandal & Bhattacharyya, 1987b).

Prolactin assay

Human seminal plasma prolactin was assayed by the Immuchem human prolactin IRMA Kit (Cat. No. 07-274102) from ICN Biomedicals, Inc. (Carson, CA 90746). This is a solid phase, two-site immunoradiometric assay in which two antibodies are used which recognize different epitopes of the same antigen. The sample (0.1 ml) was pipetted into the prolactin antibody-coated tube along with the complimentary antibody labelled with ^{125}I. After the incubation period, the tubes were emptied and washed twice to remove unbound ^{125}I-labelled antibody. The tubes were counted in a gamma counter and calibrated against human prolactin standards (0, 2.5, 5, 10, 25 and 50 ng ml^{-1}) which, in turn, had been calibrated against the WHO 75/504 reference preparation. The minimal detectable concentration was 0.25 ng per tube. The cross-reactivity with human growth hormone, which resembles prolactin structurally, was $<1.0\%$. Recoveries of added standards from normally and poorly coagulated seminal plasma were 102 and 105%, respectively. The intra-assay coefficient of variation using 50 and 100 µl seminal plasma from a pool concentration of 13.2 ng ml^{-1} was 2.1% ($n=6$). All samples were evaluated after slow thawing at 4°C from −70°C, in four sets of assays within 26 h. Prolactin concentrations were comparable in fresh ejaculates (i.e. within 20 min of emission) and in those kept for 6 h at room temperature ($n=3$).

α-Glucosidase assay

α-1,4-Glucosidase (maltase) activity was measured using the method of Chapdelaine *et al.* (1978) with a few modifications. In 1.4 ml of potassium phosphate buffer (67 mM, pH 6.8), substrate (*p*-nitrophenol-α-D-glucopyranoside, 5 mg ml^{-1}) mixture at 37°C, 0.05 ml of reduced glutathione (1 mg ml^{-1} H_2O) and 25 µl of human seminal plasma were added and incubated at 37°C for 2 h. The reaction was stopped by adding 10 ml 0.1 M sodium carbonate to the incubation medium and the intensified colour of the released *p*-nitrophenol in alkaline medium was measured spectrophotometrically at 400 nm against a reagent blank and *p*-nitrophenol standards. The intra-assay coefficient of variation of the method using 25 µl undiluted seminal plasma was 2.7% ($n=7$). The maltase activity of human seminal plasma is known to be stable on repeated ($n=4$) freezing and thawing (Chapdelaine *et al.*, 1978). All samples were assayed in four sets within 30 h after slow thawing at 4°C.

Enzyme activity was expressed as units; 1 U corresponds to 10 nmole of *p*-nitrophenol released per minute of the assay system (Casano *et al.*, 1987).

Statistical analysis

The significance of the inter- and intra-individual differences were calculated by Student's *t*-test for unpaired (Table 1) and paired (Fig. 1) data respectively (Richterich, 1969). The relationship between two variables was determined by calculation of the correlation coefficient (r) and its significance by the *t*-test.

Results

In this population, the percentage coagulum of the ejaculate varied from 0 to 100%. Based on this variation, the ejaculates were divided into four distinct groups (see Table 1), as indicated elsewhere (Mandal & Bhattacharyya, 1987c, 1989, 1990a). The samples from groups III (51–75% coagulum) and IV (76–100% coagulum) were considered as normally coagulated ejaculates, because 50–80% of the total ejaculate volume is contributed by the vesicular coagulum, about 15–40% by prostatic secretions and 5% from the testes and epididymides (Zaneveld & Tauber, 1981; Wetterauer, 1986). Consequently, the samples of groups I (0–20% coagulum) and II (21–50% coagulum) were considered as poorly and deficiently coagulated ejaculates respectively. The mean liquefaction rate curves of these four groups are presented in Fig. 1.

A highly significant and close correlation was observed between the prolactin concentration and the percentage coagulum of these ejaculates ($r = 0.686$, $n = 58$, $P<0.001$). Both the concentration and the content of prolactin in the poorly

Table 1. Prolactin level and α-glucosidase activity in seminal plasma samples (from infertile couples) exhibiting different percentage coagulum

Nature of coagulation (Percentage coagulum 4 min after emission)	Number of cases	Prolactin		α-Glucosidase*	Sperm†	
		ng ml^{-1}	ng $ejaculate^{-1}$	U ml^{-1}	$\times 10^{-6}$ ml^{-1}	Percentage motility
I Poorly coagulated 0–20%; (6.9±2.0)	15	5.2±0.48	18.2±2.23	3.6±0.80	41.0±11.9	56.1±4.2
II Deficiently coagulated 21–50%; (32.7±2.7)	10	7.6±0.72	22.7±4.39	5.0±0.59	66.5±16.9	62.2±3.2
III Normally coagulated 51–75%; (62.1±1.8)	18	8.2±0.43	26.8±2.40	3.8±0.61	55.5±13.9	57.1±4.5
IV Normally coagulated 76–100%; (89.2±2.3)	15	9.9±0.59	28.0±2.30	3.9±0.42	56.8±10.8	57.9±4.4
Significance between the groups						
I and II		$P<0.01$	NS	NS	NS	NS
I and III		$P<0.001$	$P<0.02$	NS	NS	NS
I and IV		$P<0.001$	$P<0.01$	NS	NS	NS
II and III		NS	NS	NS	NS	NS
II and IV		$P<0.025$	NS	NS	NS	NS
III and IV		$P<0.025$	NS	NS	NS	NS

Values are the mean ± SEM.

*One unit corresponds to 10 nmole of *p*-nitrophenol released per minute of the assay system.

†Excluding azoospermic cases.

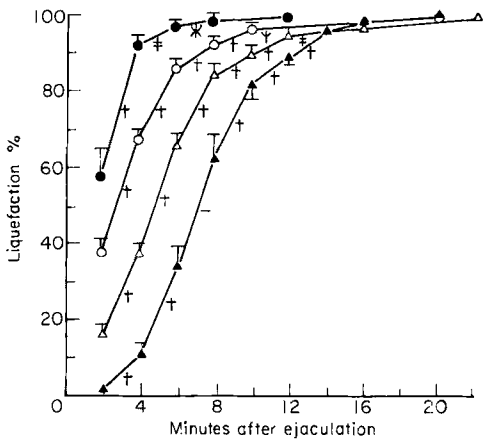

Fig. 1. Liquefaction rates of poorly coagulated (group I, $n = 15$, ●), deficiently coagulated (group II, $n = 10$, ○) and normally coagulated (group III, $n = 18$, △; and group IV, $n = 15$, ▲) human ejaculate at room temperature. Liquefied volumes were measured every 2 min after ejaculation and expressed as a percentage of the total (i.e. percentage liquefaction). Vertical bars indicate the SEM. All the intra-group differences in the percentage liquefaction values between the subsequent time points (e.g. between 2 and 4 min, between 4 and 6 min, between 6 and 8 min, etc.) were significant at $P<0.001$ (*), $P<0.005$ (†), $P<0.02$ (‡) and $P<0.05$ (ϒ) at least up to 95% liquefaction of the whole ejaculate.

coagulated group were found to be significantly lower than in the normally coagulated groups (see Table 1). A correlation coefficient of 0.407 ($n = 47$, $P<0.005$) was found between prolactin levels and the sperm concentrations of this population, excluding azoospermic samples. In presumably fertile samples (sperm count $\geq 60 \times 10^6$ ml^{-1} with $\geq 70\%$ motility, $n = 9$) and in prostatic fluid obtained from a man with congenital absence of the vasa deferentia and seminal vesicles, the prolactin concentrations were 9.2 ± 0.74 and 1.5 ng ml^{-1} respectively.

The activity of α-glucosidase demonstrated no statistically significant variations between the four groups exhibiting different degrees of coagulation (see Table 1), and no correlation was found between these two variables ($r = 0.006$, $n = 58$). A close correlation was observed between glucosidase activity and the sperm count ($r = 0.716$, $n = 47$, $P<0.001$). In presumably fertile men (sperm count $\geq 60 \times 10^6$ ml^{-1} with $\geq 70\%$ motility, $n = 9$) and in vasectomized men ($n = 4$) the enzyme activities were 5.6 ± 0.66 U ml^{-1} and 0.5 ± 0.004 U ml^{-1}, respectively.

Discussion

The concentration of prolactin observed in the normally coagulated groups (see Table 1) was similar to the samples from presumably fertile men and the normozoospermic population of Schoenfeld *et al.* (1979) (11.1 ng ml^{-1}) and Aiman *et al.* (1988) (8.3 ng ml^{-1}). The positive correlation reported herein, between seminal prolactin and the sperm concentration, is also in agreement with others (Biswas *et al.*, 1978; Aiman *et al.*, 1988). No correlation, however, exists between serum and seminal plasma prolactin levels (Aiman *et al.*, 1988). The appearance of prolactin in human seminal plasma is contributed mainly by the seminal vesicles, possibly through the accumulation of prolactin by the specific binding sites on the cell

membranes of the seminal vesicles (Barkey *et al.*, 1979; Aiman *et al.*, 1988). This has been supported by the observations of Schoenfeld *et al.* (1979) (1.4 ng ml^{-1}, $n = 10$) and our own (1.5 ng ml^{-1}, $n = 1$) where very low concentrations of prolactin in the semen of men with a congenital absence of the vasa deferentia and seminal vesicles was observed. Furthermore, Luqman & Smith (1979) showed equivalent seminal plasma prolactin concentrations before (12.7 ± 6.1 ng ml^{-1}) and after (11.4 ± 3.2 ml^{-1}) vasectomy, and by the immunohistochemical localization of prolactin only in the (sub-epithelial stroma of the) seminal vesicles but not in other parts of the genital tract (Wahlstrom & Ranta, 1983).

The significant decrease in the concentration and total content of prolactin in the group with poorly coagulated ejaculates, compared with the normally coagulated ejaculates, suggests that the mechanism for vesicular accumulation of this hormone in abnormally coagulating samples could be inefficient. In addition, these variations can be used to explain our previous observation that sperm motility decreases rapidly in poorly coagulated samples (Mandal & Bhattacharyya, 1988a), as the addition of prolactin antiserum has been reported to decrease sperm motility (Luqman *et al.*, 1981). The close positive correlation between the prolactin level and the percentage coagulum indicates that this pituitary hormone may be involved in coagulum formation of human semen and so in the activity of the seminal vesicles. This interpretation is supported by a recent observation (Gonzales *et al.*, 1989) which demonstrated that seminal prolactin was decreased in subjects with lower levels of seminal fructose. Moreover, the observed correlation may, at least partially, explain the decreased production of vesicular fructose, amino sugar, choline and phosphate in the seminal plasma of poorly coagulated samples (Mandal & Bhattacharyya, 1985c, 1986b, 1987b, 1990a), because it is known that prolactin directly and in synergy with testosterone influences the secretory activity of the accessory sex glands (Mann & Lutwak-Mann, 1981). In view of the biological actions of prolactin on the male accessory organs (Ensor, 1978) and the present study's findings, further studies on prolactin may be helpful in elucidating the factors involved in human seminal coagulum formation.

Comparison of the mean seminal levels of α-glucosidase in vasectomized and presumably fertile men confirms its prevalent epididymal origin (91%), as a conclusion reached by others (Tremblay *et al.*, 1982; Casano *et al.*, 1987). The characteristic positive correlation of this epididymal marker with the sperm count (Tremblay *et al.*, 1979, 1982; Casano *et al.*, 1987) is also confirmed in the present study ($r = 0.716$, $P < 0.001$, $n = 47$). This epididymal index revealed no relationship with the degree of coagulation of the ejaculates when glycerylphosphorylcholine, another less specific epididymal marker component in human semen showed a positive correlation (Mandal & Bhattacharyya, 1989). It is worthwhile mentioning that the content of glycerylphosphorylcholine was determined in terms of glycerol (White, 1959), whereas human semen contains equimolar amounts of glycerol and glycerylphosphorylcholine (Frenkel *et al.*, 1974); only 50% of the seminal plasma glycerylphosphorylcholine content was found to be of epididymal origin while the rest was produced mainly by the seminal vesicles (Mandal *et al.*, 1989), whose decreased secretory activity has been amply observed in poorly coagulated ejaculates (Mandal & Bhattacharyya, 1985a, c; 1986b; 1987b). Considering these facts in relation to our previous and present results on the epididymal markers, we do not suggest a

significant relationship between the degree of coagulation of human ejaculates and epididymal functions unlike that of the seminal vesicles (Mandal & Bhattacharyya, 1985c; 1986b) and prostate (Mandal & Bhattacharyya, 1987b).

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