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·*Original Article*·

Age-related changes in seminal polymorphonuclear elastase in men with asymptomatic inflammation of the genital tract

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Abstract

Aim: To investigate age-related inflammatory events in the male genital tract. **Methods:** In a total of 4 265 randomly collected patients attending the andrological outpatient clinic of the Center for Dermatology and Andrology, University of Giessen, Germany, ejaculate volume, pH-value, sperm concentration, total and progressive sperm motility, concentration of polymorphonuclear (PMN) elastase, number of peroxidase-positive cells and fructose were measured and correlated with patient's age. **Results:** While ejaculate volume, motility and fructose all correlated negatively with age, sperm concentration, PMN elastase and the pH-value showed a positive correlation. The prevalence of male genital tract inflammation (as defined by PMN elastase > 250 ng/mL) and its severity increased significantly. PMN elastase did not correlate with sperm motility. Fructose as a marker of seminal vesicle function showed a significant negative relationship with the PMN elastase levels, the number of peroxidase-positive cells and sperm motility. **Conclusion:** The significant increases of PMN-elastase levels as marker of male genital tract inflammation in older men appear to be indicative of age-related changes in local immunoregulatory mechanisms. Because there is no association of PMN elastase with sperm motility, a direct inhibitory effect of this enzyme can be excluded. *(Asian J Androl 2007 May; 9: 299–304)*

Keywords: aging men; male genital tract inflammation; polymorphonuclear elastase; leukocytes; infertility; human semen

1 Introduction

Male genital tract inflammation is considered a major contributing factor to infertility. The majority of patients, however, do not notice any clinical symptoms. Therefore, an accurate andrological examination according to World Health Organization (WHO) guidelines is mandatory [1]. To prove genital tract inflammation and respective semen patterns, several diagnostic methods have been described, including the determination of peroxidase-positive cells in the ejaculate [2] or seminal polymorphonuclear (PMN) elastase [3]. However, the absence of leukocytes in semen does not exclude the possibility of infections or inflammatory reactions of the male genital tract. Thus, the criterion "leukocytospermia" as defined by WHO (more than 10^6 leukocytes/mL ejaculate) is not

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accurate and is still a matter of ongoing debate [4, 5]. Even low numbers of macrophages in semen may indicate inflammatory disorders of the epididymis and significantly impaired sperm functions and thus fertility [6]. In order to improve semen analysis in this respect, the immunologic measurement of PMN elastase was introduced [3] and has been proven a reliable marker of silent male genital tract inflammation [7, 8].

Aging in men does not only decrease serum testosterone levels [9], but may also affect fertility [10, 11]. Moreover, the human immune system undergoes age-related changes that have been associated with increased morbidity and mortality as a result of infections, malignancies, or autoimmune diseases [12]. Notably, immunosenescence is a complex process involving multiple reorganizational and developmentally regulated changes, rather than a simple unidirectional decline in all functions [13]. One of the basic mechanisms proposed to explain these aging processes is the free radical theory [14]. It appears that almost every component of the immune system undergoes age-dependent changes resulting in an imbalance between oxidative stress and antioxidative defense, which will consequently lead to an increased production of reactive oxygen species (ROS) by phagocytes [12].

Because there are initial reports indicating increased inflammatory events in the human genital tract in aged men [7, 15] and spermatozoa are particularly susceptible to oxidative stress caused by leukocytes, it was the aim of this study to get further insight into the possible mechanism of decreasing reproductive functions in older men.

2 Material and methods

In this retrospective study, a total of 4 265 consecutive patients attending the andrological outpatient clinic for fertility problems between January 1998 and January 2000 (17–66 years old) were analyzed for the following parameters: age (n = 3 916), volume of the ejaculate (n = 4 121), pH-value (n = 4 113), sperm concentration (n = 3 916), total motility (n = 4 265), progressive motility (n = 4 265), concentration of the polymorphonuclear granulocyte elastase (PMN elastase) (n = 2 115), number of peroxidase-positive cells (n = 1 507) and the concentration of fructose (n = 1 488) as marker of seminal vesicle function. A complete set of data of all parameters was available for 1 942 patients, which includes up to five consecutive measurements per patient.

Semen analysis including the above-mentioned parameters was performed according to WHO guidelines [2]. PMN elastase was determined in cell-free seminal plasma as described by Jochum *et al*. [3], using an enzyme-linked immuno-absorbent assay provided by DPC Biermann (Bad Nauheim, Germany). Patients whose ejaculates showed PMN elastase concentrations > 250 ng/mL were regarded as having a genital tract inflammation and those showing PMN elastase levels >1 000 ng/mL as having a severe inflammation [3].

All statistical calculations (mean, median and Spearman's Rank correlation) were performed after testing for normal distribution of the data by means of the Kolmogorov-Smirnov test. In addition, the data set was categorized in three subgroups according to age (subgroup 1, ≤ 30 years; subgroup 2, 31–45 years; subgroup 3, > 45 years), medians and the differences between the subgroups were calculated by means of the Mann-Whitney *U*-test and H-test according to Kruskal-Wallis. All calculations were carried out with MedCalc (Version 9.2, MedCalc Software, Mariakerke, Belgium). $P < 0.05$ was considered as significantly different.

3 Results

The summary statistics of all analyzed parameters are compiled in Table 1. The high variation of values, reflected by the high standard deviation (SD), is obvious as it is expected for biological parameters. In Table 2, the age of the patients is correlated with the results of basic semen analysis and biochemical parameters. Significant decreases of the values for the ejaculate volume, motility, and for the concentration of fructose in elderly men was revealed and the decline confirmed by means of the H-test according to Kruskal-Wallis ($P < 0.004$) (Table 2). The decreases per year ranged between –0.70% for total motility and –0.84% for the ejaculate volume. However, significantly increasing values in older men were found for sperm concentration, PMN elastase and the pH-value, while the number of peroxidase-positive cells remained unchanged (Table 2). The general prevalence of male genital tract inflammation as determined by means of PMN elastase (> 250 ng/mL) and the peroxidase stain of leukocytes (> 10^6/mL) was 30.1% and 36.7%, respectively.

When focusing on male genital tract inflammation, not only a significantly higher prevalence was obvious in men older than 30 years, but also the seminal concentra-

tion of PMN elastase increased (Table 3). The concentration of PMN elastase between subgroups 1, 2 and 3, respectively, differed significantly (Figure 1). However, although PMN elastase and the number of peroxidase-

Table 1. Summary statistics of the parameters analyzed in the study. PMN, polymorphonuclear.

Parameters	*n*	Mean ± SD	Range	Median
Age (years)	3916	33.9 ± 5.9	17–66	33.0
Sperm concentration (million/mL)	3916	49.7 ± 73.8	0–740	21.8
Volume of ejaculate (mL)	4121	3.8 ± 1.9	0.01–14	3.5
Total motility (%)	4265	40.8 ± 17.5	0–86	42.5
Progressive motility (%)	4265	31.8 ± 18.2	0–80	32.0
PMN elastase (ng/mL)	2115	260.3 ± 381.3	0–4285	103.0
No. peroxidase-positive cells (million/mL)	1507	1.4 ± 2.6	0–30	0.5
pH-value	4113	7.46 ± 0.27	4–8	7.5
Fructose (μmol/mL)	1488	15.7 ± 7.6	0.2–37.3	14.6

Table 2. Correlation of age with different semen parameters. PMN, polymorphonuclear.

Parameter	*n*	Spearman's ρ	*P*	H-test after dividing into three subgroups (*P*)	Change per year (%)
Sperm concentration	3915	0.095	< 0.0001	< 0.0001	1.84
Volume of ejaculate	3866	–0.079	< 0.0001	< 0.0001	–0.84
Total motility	3127	–0.061	0.0007	< 0.0001	–0.70
Progressive motility	3127	–0.058	0.0012	< 0.0001	–0.77
PMN elastase	1942	0.086	0.0002	0.0003	0.89
Peroxidase-positive cells	1413	0.015	0.5628	0.4960	–
pH-value	3858	0.029	0.0679	0.0084	0.01
Fructose	1261	–0.084	0.0030	0.0037	–0.80

Table 3. Concentration of seminal polymorphonuclear (PMN) elastase (ng/mL) in 1 942 patients and age-dependent subgroups. Significant increases of the PMN elastase and the incidence of male genital tract inflammation in patients older than 30 years are obvious. The PMN elastase concentrations between subgroups 1, 2 and 3 differ significantly ($P = 0.0001$, between subgroups 1 and 2; $P = 0.4653$, between groups 2 and 3; $P = 0.0184$, between groups 1 and 3, see also Figure 1). SD, standard deviation.

	All	Subgroup 1 (≤ 30 years)	Subgroup 2 (31–45 years)	Subgroup 3 (> 45 years)
n	1942	514	1363	65
Minimum	0.0	3.0	0.0	9.0
Maximum	4285	1750.0	4285.0	989.0
Mean	266.7	213.9	288.1	236.2
Median	104.0	80.0	117.0	145.0
SD	389.0	321.9	414.9	249.5
Patients with genital tract inflammation (PMN elastase >250 ng/mL, *n* = 582)	584.0	128.0	437.0	19.0
Prevalence (%)	30.1	24.9	32.1	29.2
Patients with severe genital tract inflammation (PMN elastase >1000 ng/mL, *n* = 116)	116.0	19.0	97.0	0
Prevalence (%)	5.9	3.7	7.1	0

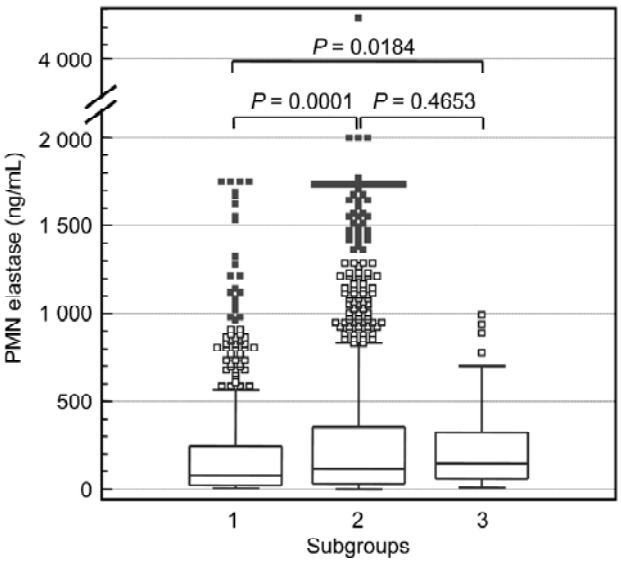

Figure 1. Age-dependent concentration of seminal polymorphonuclear (PMN) elastase. Men younger than 30 years (subgroup 1) show significantly lower seminal PMN elastase concentrations than older men (subgroup 2, 31–45 years; subgroup 3, older than 45 years). No difference can be seen between subgroups 2 and 3 (see also Table 3). n = 1 942. Squares indicate data points outside the Box-and-Whisker-Plot.

positive cells correlated significantly in an overall analysis (n = 884; r = 0.649; P < 0.0001), age-related changes in the number of peroxidase-positive cells could not be observed (Table 2). In addition, significant negative correlations were found for the parameters of inflammation with the ejaculate volume (Table 4). The pH-value, which is also indicative of male genital tract inflammation, correlated positively with PMN elastase (n = 2 111; r = 0.168; P < 0.0001) and the number of peroxidase-positive cells (n = 1 507; r = 0.060; P = 0.0200), although the latter association was markedly weaker. Both, PMN elastase and the number of peroxidase-positive cells showed no relationship with total motility (n = 1 773; r = 0.029; P = 0.2191 and n = 1 380; r = 0.002; P = 0.9442) and progressive motility (n = 1 767; r = 0.004; P = 0.8590 and n = 1 383; r = –0.002; P = 0.9462) respectively. This result for the correlation between PMN elastase and motility was not only obvious for all measurements performed but also for all single patients with repeated measurements (n = 264) (data not shown).

The functional marker of the seminal vesicles, fructose, showed a significant negative relationship with

Table 4. Correlation of the ejaculate volume with parameters indicating inflammation of the genital tract. PMN, polymorphonuclear.

Parameter	n	r	P
PMN elastase	2113	–0.146	< 0.0001
No. peroxidase-positive cells	1507	–0.065	0.0116
pH-value	4112	–0.193	< 0.0001

PMN elastase (n = 1 291; r = –0.055; P = 0.0495) and the number of peroxidase-positive cells (n = 619; r = –0.211; P < 0.0001), but a weak positive correlation with the pH (n = 1 487; r = 0.070; P = 0.0072) and the ejaculate volume (n = 1 487; r = 0.222; P < 0.0001). Its correlation with total (n = 1 213; r = –0.061; P = 0.0336) and progressive motility (n = 1 216; r = –0.062; P = 0.0301) was negative.

4 Discussion

The age-dependent decrease of male reproductive functions accompanied by a significant decrease of the ejaculate volumes is a well-known phenomenon that is caused by the significant decline of the concentration of free testosterone and has been repeatedly described [9, 10]. With regard to genital tract infection or inflammation among aging males, however, there are only two short reports describing an age-dependent increase of male genital tract inflammation as determined by seminal PMN elastase [7] or WHO criteria of “male accessory gland infection” including positive aerobic or anaerobic bacteriological culture [15] in relatively small populations of 312 and 388 patients, respectively.

Infection and inflammation of the male reproductive tract are accepted as important etiological factors of male infertility [1]. It should be noted, however, that a correct diagnosis is hampered by imprecise definitions and the asymptomatic course of these disorders in the majority of patients. Thus, the reported prevalence of genital tract inflammation varies considerably and is, of course, dependent on the method and the cut-off values used [5]. However, immunologic measurement of PMN elastase has been proven a reliable marker of silent male genital tract inflammation [3, 7, 8].

In this report, a large population study of 1 942 patients in which PMN elastase was analyzed and could be correlated to other parameters, we can clearly confirm the age-dependent increase of the prevalence of male

genital tract inflammation reflected by seminal PMN elastase concentrations. The overall frequency of male genital tract inflammation as detected by PMN elastase levels higher than 250 ng/mL [3] and leukocytospermia (>1 million leukocytes/mL ejaculate; [2]) was 30.1% and 34.6%, respectively. While leukocytospermia was more frequent than that in previously published reports, the prevalence of elevated PMN elastase concentrations in semen corresponded with recent reports [2, 3, 7, 8]. In addition to the increasing incidence of the inflammation, we found an increased severity of male genital tract infections in older men.

Despite a strong positive correlation of the PMN elastase with the number of peroxidase-positive cells in the ejaculate, the latter did not show any relationship to age. Thus, the increase of PMN elastase in older men might be explained by the observed age-related decrease of the ejaculate volume, which has also been reported previously [10]. Considering that the seminal vesicle secretions contribute most to the ejaculate volume, its decline can be due to an age- and therefore testosterone-dependent seminal vesicle insufficiency [16] and would be consistent with the negative relationship of fructose as marker of seminal vesicle function with age.

The increased levels of PMN elastase in elderly men can also indicate inflammation of the male accessory glands [17], thus leading to reduced ejaculate volumes. This hypothesis would be in agreement with our observation that seminal volume is negatively correlated with all parameters of inflammation investigated. Consistently, fructose showed an inverse correlation with PMN elastase and the number of peroxidase-positive cells, suggesting an adverse effect of inflammation on the seminal vesicle. These results also confirm data reported by Wolff *et al*. [18].

However, the age-related increase of the seminal concentration of PMN elastase might reflect changes in local immune mechanisms as part of the general immunosenescence. Dysfunction of the aging immune system comprises adaptive immune responses and innate defence mechanisms, and is thought to contribute to an increased incidence of infections and chronic inflammatory disease [7, 12, 15]. At the cellular level, lymphocytes, phagocytes, and other immune cells undergo age-related alterations of their functions. For example, the production of pro-inflammatory cytokines is significantly increased [19]. Thus, it would be plausible that the prevalence of male genital tract infections and/or inflammation increases with aging.

Notably, immune cells including macrophages, mast cells, and lymphocytes and their products are not only encountered in the testis but also regular components of the epididymis and excurrent ductal system [20]. Age-related changes in leukocyte activity might contribute to high levels of the PMN elastase in seminal plasma. In addition, inflammatory deterioration of accessory gland function with decreased secretions could result in elevated levels of the enzyme in the first instance. In addition, the aforementioned age-related change in leukocyte activity might contribute to the high levels of enzyme in seminal plasma.

The deleterious influence of male genital tract infections/inflammation and thus leukocytes on the excurrent ductal system [21] and sperm functions like motility, acrosomal function or sperm DNA fragmentation [22, 23] must not be underestimated. However, a direct influence of this enzyme on sperm function seems rather unlikely as PMN elastase appeared to have no influence on sperm motility. This observation is consistent with data reported by Maegawa *et al*. [24] who suggested that a sufficient amount of the secretory leukocytes protease inhibitor in seminal plasma prevents spermatozoa from being attacked by elastase. Therefore, other leukocyte-derived inflammatory factors like ROS or pro-inflammatory cytokines [25] should also be considered with regard to their impact on organ and sperm functions. In contrast, Kopa *et al*. [8] reported a significant negative correlation of seminal PMN elastase concentrations and sperm motility.

In fact, the negative influence of peroxidase-positive cells on progressive motility and sperm DNA fragmentation seems to be mediated by ROS as reported previously [5]. Thus, the age-related decrease in sperm motility is not necessarily associated with the increased seminal concentration of PMN elastase, but rather linked to a dysfunctional epididymis in older men. The latter causes a disturbed removal of the element zinc from the sperm flagella during epididymal maturation, eventually leading to poor motility [11]. Alternatively, an increased imbalance between oxidative stress on the one hand, and a lack of antioxidant capacity on the other hand, could cause or at least contribute to the documented declines in sperm function.

In conclusion, we found a significant increase of PMN elastase levels in older men while the number of peroxidase-positive cells in the ejaculate did not change,

which is most probably related to immunosenescence. Because there is no association of PMN elastase with sperm motility, a direct effect of this enzyme can be excluded. Consequently, the age-dependent decrease of sperm motility is most probably associated with altered epididymal zinc elimination from the spermatozoa as proposed in an earlier study [11], or ROS might play a role in the pathological mechanism. This is the subject of further investigation.

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