

Seminal mast cells in infertile asthenozoospermic males

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Keywords

Male infertility—mast cells—semen—sperm motility—spermatozoa

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Accepted: July 25, 2007

Summary

This work aimed to assess the possible association between the presence of seminal mast cells and asthenozoospermia. One hundred and seventy-six male subjects were investigated: group (Gr)1 ($n = 46$) normozoospermic fertile controls, Gr2 ($n = 62$) idiopathic asthenozoospermia, Gr3 ($n = 32$) asthenozoospermia with scrotal varicocele and Gr4 ($n = 36$) asthenozoospermia with leucocytospermia. Four smear slides were prepared for each semen sample to be stained with toluidine blue–pyronin to detect mast cells. A significant increase was shown in mast cell-positive samples among varicocele-associated and idiopathic asthenozoospermic patients in comparison with fertile controls. Seminal mast cells were also detected at higher frequency among smokers and in age group over 40 years. It is concluded that mast cells and their products may play a pivotal role in the pathogenesis of asthenozoospermia, possibly proposing a new goal for medical treatment of infertile males to pursue. In addition, this concept may in a way detain smoking as a cause of male infertility considering the clear abundance of mast cells in semen samples of smokers.

Introduction

Mast cells are commonly found in connective tissue or in mucosa of many organs including skin, airways, digestive tract, testis, epididymis and the female reproductive tract. They are considered as part of the immune system and are suggested to be generated by different precursor cells in the bone marrow (Metcalf *et al.*, 1999). They are divided into two subtypes based on differences in their neutral serine protease content; mast cells containing tryptase (MCT) and mast cells containing both tryptase and chymase (Welle, 1997; Yong, 1997). In mammalian testis, MCT is a potent mitogen for fibroblast that can enhance the synthesis of collagen with subsequent fibrosis and thickening of the tubular wall (Guarch *et al.*, 1992).

Several reports have associated increased number of mast cells in the testes and male infertility (Agarwal *et al.*, 1987; Hashimoto *et al.*, 1988; Nagai *et al.*, 1992; Roaiah *et al.*, 2007). Abnormal spermatogenesis is associated with an increased number of testicular macrophages and an increased numbers of B and T lymphocytes; the former is associated with increased production of tryptase enzyme which is a potent mitogen for the fibroblast that can

enhance their chemotaxis and activation resulting in tubular fibrosis (Apa *et al.*, 2002). Also, increased number of mast cells is responsible for the marked deposition of collagen and testicular fibers seen in testis with abnormal spermatogenesis (Hussein *et al.*, 2005).

Nagai *et al.* (1992) found that the number of mast cells was increased and the ratio of mast cell subtypes was changed in idiopathic azoospermia and oligozoospermia. Therefore, mast cell blockers have been empirically used in the treatment of male infertility with some reports of significant improvement in semen quality as well as spontaneous pregnancies (Yamamoto *et al.*, 1995; Matsuki *et al.*, 2000; Hibi *et al.*, 2002). In contrast, other authors showed that mast cell blockers had no benefit in treating infertile men (Cayan *et al.*, 2002).

The aim of this work was to assess seminal mast cell content in asthenozoospermic infertile males.

Materials and methods

One hundred and seventy-six men were selected prospectively after consent from the Andrology Outpatient Clinic of the University Hospital after Institutional Review

Board approval. They were divided into group (Gr)1 ($n = 46$) fertile normozoospermic controls, Gr2 ($n = 62$) idiopathic asthenozoospermia (sperm motility $<50\%$), Gr3 ($n = 32$) asthenozoospermia with scrotal varicocele and Gr4 ($n = 36$) asthenozoospermia with leucocytospermia. A detailed medical history was taken and physical examination with scrotal duplex for varicocele diagnosis was performed for the studied cases. Ejaculates were obtained in the early morning (7:00–9:30 hours) after 4 days of sexual abstinence. The samples were examined immediately conventionally after liquefaction according to the guidelines of WHO (1999) (normally, sperm count $>20 \times 10^6$ sperm ml^{-1} , sperm motility $>50\%$, abnormal sperm morphology $<70\%$, vitality $>75\%$ and leucocytes $<10^6$ ml^{-1}). Semen samples were verified after at least two different analyses.

To evaluate mast cells, four semen slides were prepared for each case. The slides were fixed through air drying and stained with 1% toluidine blue–pyronine (pH 4). Then, they were evaluated under immersion objective. If mast cells were detected in the slides, the cases were considered as mast cells positive (+ve) and if not found, as mast cells negative (–ve) (Fig. 1).

Paired Student's *t*-test was used to compare parametric groups. Qualitative variable expressed as percentages was compared in different groups using the chi-squared test comparing the frequencies in different groups with theoretical values under the null hypothesis. They were considered statistically significant when $P < 0.05$.

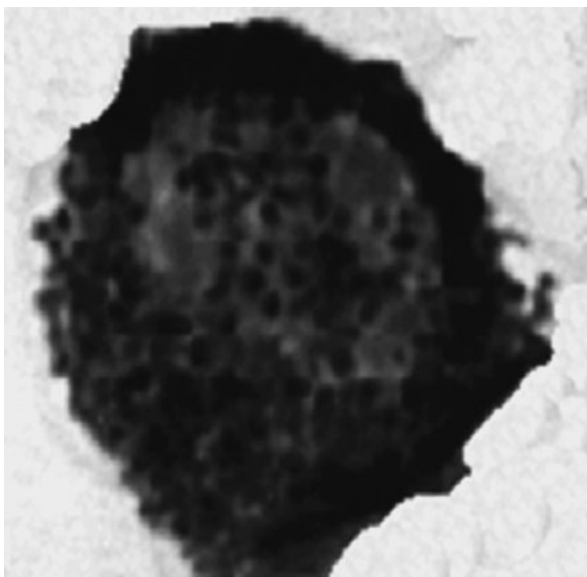


Fig. 1 Mast cell in semen stained by toluidine blue stain.

Results

Mast cells were detected in 100 cases out of 176 (56.4%); 16 in fertile control group (34.8%), 40 in idiopathic asthenozoospermic group (64.5%), 22 in asthenozoospermia with varicocele group (68.75%) and 22 in asthenozoospermia with leucocytospermia group (61.1%). Mast cells (+ve) cases were significantly higher in idiopathic asthenozoospermia and varicocele-associated asthenozoospermia groups in comparison with the controls. Although higher in asthenozoospermia with leucocytospermia group, the difference in comparison with control group was non-significant (Table 1). Mast cells (+ve) cases were shown to be significantly higher in those >40 years (32 cases) compared with those <40 years (18 cases). Also, mast cells (+ve) cases were significantly higher in smokers (33 cases) compared with non smokers (17 cases) (Table 2).

Discussion

In the present study, a significant increase in mast cells was detected among idiopathic asthenozoospermic as well as varicocele-associated asthenozoospermic patients compared with controls. A similar increase was noticed in cases of asthenozoospermia associated with leucocytospermia, though nonsignificant. All these categories of asthenozoospermia were documented to be associated with increased seminal reactive oxygen species (ROS) and decreased seminal antioxidants (Lewis *et al.*, 1995; Mostafa *et al.*, 2001; La Vignera *et al.*, 2006). The relationship between ROS and seminal mast cells was not discussed before, but Santos *et al.* (2000) suggested that ROS can act as stimulators of mast cell degranulation in burns. Renke *et al.* (2006) reported that mast cells generate intracellular ROS in response to stimulation with divergent physiologically relevant stimulants.

Few studies have evaluated the relationship between isolated asthenozoospermia and mast cells. Worth mentioning, Weidinger *et al.* (2003) showed, by immunoelectronmicroscopy, proteinase-activated receptor-2 (PAR-2) in the membranes of the acrosomal region and midpiece of human spermatozoa. These PAR-2 were functional, as exposure of spermatozoa from healthy men with regular standard semen parameters to human recombinant tryptase significantly decreased motility in a dose- and time-dependent fashion. In a study by Cincik & Sezen (2003), semen from 400 randomly selected men was examined for mast cell content in relation to sperm concentration, motility and morphology. 21.5% mast cell-positive cases showed relative decline in sperm parameters, with statistical significance only evident regarding progressive motility, in comparison with mast cell-negative cases.

	Group 1	Group 2	Group 3	Group 4
Mast cells (-ve)	30 (65.2%)	22 (35.5%)	10 (31.25%)	14 (38.9%)
Mast cells (+ve)	16 (34.8%)	40 (64.5%)	22 (68.75%)	22 (61.1%)
Chi-square		4.68	4.36	2.81
<i>P</i>		<0.05*	<0.05*	>0.05

P comparison with controls.

*Significant.

	<40 years	>40 years	Nonsmokers	Smokers
Mast cells (-ve)	23 (56.1%)	15 (31.9%)	24 (58.5%)	14 (29.8%)
Mast cells (+ve)	18 (43.9%)	32 (68.1%)	17 (41.5%)	33 (70.2%)
Chi-square		5.22		7.38
<i>P</i>		<0.05*		<0.01*

*Significant.

Table 1 Seminal mast cells in different studied groups (*n*, %)

Table 2 Seminal mast cells relationship with age and smoking

Nevertheless, medical literature does bear reports on the relationship between other aspects of male infertility and mast cells. Hussein *et al.* (2005) revealed that increased number of mast cells in testicular tissues was responsible for the marked deposition of collagen in the seminiferous tubules of azoospermic patients with spermatogenic arrest and Sertoli-cell only syndrome. In the same line, Hashimoto *et al.* (1988) reported an increase in intra-testicular mast cell content in testicular tissue obtained from 16 patients with idiopathic oligozoospermia. Nagai *et al.* (1992) have also shown that the number of mast cells in testicular tissue was increased and the ratio of mast cell subtypes (chymase and tryptase) was changed in idiopathic azoospermia and oligozoospermia.

We observed that mast cell-positive cases were significantly higher among older subjects, in contrast to mast cell-negative cases. This may point to an ageing process being a possible mechanism by which mast cells contribute to infertility. This finding supports the evidence proposed by Cincik & Sezen (2003) showing that the age of mast cell-positive cases were higher than those of mast cell-negative cases.

Another observation was that seminal mast cells showed higher frequency among smokers compared with nonsmokers, suggesting an aetiological relationship between smoking and mast cell abundance in infertile patients, and therefore an indirect relationship between smoking and infertility. This supports the studies that establish smoking as having an adverse effect on fertility, especially on progressive sperm motility, irrespective of total amount of cigarettes smoked per day (Hassa *et al.*, 2006; Mostafa *et al.*, 2006).

Some of the previous therapeutic trials with mast cell blockers went along with our findings, and some did not.

Yamamoto *et al.* (1995) prescribed randomly mast cell blocker (tranilast) or placebo for 3 months infertile males with severe idiopathic oligozoospermia, showing significant improvement in semen parameters and 28.6% of pregnancy rate in the treated group with no improvement in the placebo group. Matsuki *et al.* (2000) investigated the effect of ebastine, a mast cell blocker, on semen quality in idiopathic oligozoospermic men, reporting that 66.7% of the patients had improved semen quality and 20% achieved pregnancy with their partners within 6 months of treatment. In contrast to previous studies, Cayan *et al.* (2002) found that semen parameters of infertile men did not change significantly after mast cell blocker treatment (fexofenadine) and no one achieved spontaneous pregnancy.

It is concluded that mast cells and their products may play a pivotal role in the pathogenesis of asthenozoospermia, possibly proposing a new goal for medical treatment of infertile males to pursue. In addition, this concept may in a way detain smoking, as a cause of male infertility considering the clear abundance of mast cells in semen samples of smokers.

References

- Agarwal S, Choudhury M, Banerjee A (1987) Mast cells and idiopathic male infertility. *Int J Fertil* 32:283–286.
- Apa DD, Cayan S, Polat A, Akbay E (2002) Mast cells and fibrosis on testicular biopsies in male infertility. *Arch Androl* 8:337–344.
- Cayan S, Apa DD, Akbay E (2002) Effect of fexofenadine, a mast cell blocker, in infertile men with significantly increased testicular mast cells. *Asian J Androl* 4:291–294.
- Cincik M, Sezen SC (2003) The mast cells in semen: their effects on sperm motility. *Arch Androl* 49:307–311.

- Guarch R, Pesce C, Puras A (1992) A quantitative approach to the classification of hypospermatogenesis in testicular biopsies for infertility. *Hum Pathol* 23:1032–1037.
- Hashimoto J, Nagai T, Takaba H, Yamamoto M, Miyake K (1988) Increased mast cells in the limiting membrane of seminiferous tubules in testes of patients with idiopathic infertility. *Urol Int* 43:129–132.
- Hassa H, Yildirim A, Can C, Turgut M (2006) Effect of smoking on semen parameters of men attending an infertility clinic. *Clin Exp Obstet Gynecol* 33:19–22.
- Hibi H, Kato K, Mitsui K, Taki T, Yamada Y, Honda N, Fukatsu H, Yamamoto M (2002) Treatment of oligoasthenozoospermia with tranilast, a mast cell blocker, after long-term administration. *Arch Androl* 48:451–459.
- Hussein MR, Abou-Deif ES, Bedaiwy MA, Said TM, Mustafa MG, Nada E, Ezat A, Agarwal A (2005) Phenotypic characterization of the immune and mast cell infiltrates in the human testis shows normal and abnormal spermatogenesis. *Fertil Steril* 83:1447–1453.
- La Vignera S, Calogero AE, Cannizzaro MA, Vicari E (2006) Mono or bilateral inflammatory postmicrobial prostatovesiculourethritis: differences in semen parameters and reactive oxygen species production. *Minerva Endocrinol* 31:263–272.
- Lewis SE, Boyle PM, McKinney KA, Young IS, Thompson W (1995) Total antioxidant capacity of seminal plasma is different in fertile and infertile men. *Fertil Steril* 64:868–870.
- Matsuki S, Sasagawa I, Suzuki Y, Yazawa H, Tateno T, Hashimoto T, Nakada T, Saito H, Hiroi M (2000) The use of ebastine, a mast cell blocker, for treatment of oligozoospermia. *Arch Androl* 44:129–132.
- Metcalfe DD, Baram D, Mekori YA (1999) Mast cells. *Physiol Rev* 77:1033–1079.
- Mostafa T, Anis TH, El-Nashar A, Imam H, Othman IA (2001) Varicolectomy reduces reactive oxygen species levels and increases antioxidant activity of seminal plasma from infertile men with varicocele. *Int J Androl* 24:261–265.
- Mostafa T, Tawadrous G, Roaih MMF, Amer M, Ashour S, Aziz A (2006) Effect of smoking on seminal plasma ascorbic acid in infertile and fertile males. *Andrologia* 38:221–224.
- Nagai T, Takaba H, Miyake K, Hirabayashi Y, Yamada K (1992) Testicular mast cell heterogeneity in idiopathic male infertility. *Fertil Steril* 57:1331–1336.
- Renke J, Popadiuk S, Wozniak M, Szlagaty-Sidorkiewicz A, Hansdorfer-Korzon R (2006) Mast cells, their adenosine receptors and reactive oxygen species in chronic inflammatory pathologies of childhood. *Przegl Lek* 63:554–556.
- Roaiah MMF, Khatab H, Mostafa T (2007) Mast cells in testicular biopsies of azoospermic men. *Andrologia* 39:185–189.
- Santos FX, Arroyo C, Garcia I, Blasco R, Obispo JM, Hamann C, Espejo L (2000) Role of mast cells in the pathogenesis of postburn inflammatory response: reactive oxygen species as mast cell stimulators. *Burns* 26:145–147.
- Weidinger S, Mayerhofer A, Frungieri MB, Meineke V, Ring J, Kohn FM (2003) Mast cell-sperm interaction: evidence for tryptase and proteinase-activated receptors in the regulation of sperm motility. *Hum Reprod* 18:2519–2524.
- Welle M (1997) Development, significance, and heterogeneity of mast cells with particular regard to the mast cell-specific proteases chymase and tryptase. *J Leukoc Biol* 61:233–245.
- WHO (1999) Laboratory Manual for the Examination of Human Semen and Sperm–Cervical Mucus Interaction, 4th edn. Cambridge University Press, Cambridge.
- Yamamoto M, Hibi H, Miyake K (1995) New treatment of idiopathic severe oligozoospermia with mast cell blocker: results of a single blind study. *Fertil Steril* 64:1221–1223.
- Yong LC (1997) The mast cell: origin, morphology, distribution and function. *Exp Toxicol Pathol* 49:409–424.