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The Effect of Unilateral Varicocele on the Contralateral Testicular Histo-Morphology and Function in *Rattus norvegicus*

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Experimental animal models have been extensively used to assess the effect of unilateral varicocele on ipsilateral testicular histology and function. There is therefore an enormous body of evidence on the detrimental effects of varicocele on the affected testis. A vacuum however still exists in our knowledge of the effects of unilateral varicocele on the contralateral testicular morphology and function. The present study evaluated the effect of left unilateral varicocele on the right testicular anatomy and physiology in Sgrague-Dawley rats (*Rattus norvegicus*). Fifty five immature rats were divided into two groups (group A, 45 rats and group B, 10 rats). Group A rats were rendered experimentally varicocelized by the complete ligation of their left main spermatic veins. Group B rats were sham operated to serve as control. Sixteen weeks after varicocele induction, bilateral testicular weight, bilateral testicular volume, bilateral caudal epididymal sperm characteristics, bilateral testicular histomorphometry and fertilizing capacity were all tested. The results show that left testicular weight and volume were significantly lower ($p<0.05$) than the right testicular weight and volume in varicocelized rats. The right testicular weights and volumes in varicocele rats were however also significantly lower ($p<0.05$) compared to the testicular weights and volumes of the control group. Further the sperm content and percentage motility were significantly lower ($p<0.05$) in the left epididymides than the right epididymides of the varicocele rats. However, the caudal epididymal sperm concentration and percentage sperm motility were significantly lower ($p<0.05$) bilaterally in the varicocele group compared to the control rats. Histomorphological profiles of the groups of animals parallel the sperm parameter findings. Present results indicate a bilateral derangement of testicular morphology and function with unilateral varicocele.

Key words: Varicocele, testis, morphology, function, *Rattus norvegicus*

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INTRODUCTION

Varicocele is the abnormal tortuosity and dilatation of the veins of the pampiniform plexus that drain the testis. Varicocele occurs in 15% of the general population and it is the most prevalent etiology of secondary male infertility (Gorelick and Goldstein, 1993). The pathophysiology of testicular damage in varicocele has not been completely understood. Currently, research into the pathophysiology of varicoceles has focused on three main areas. These are oxidative-related damage, tissue hypoxia and hormonal inbalances.

Varicocele may be associated with an increase in the Reactive Oxygen Species (ROS) generation and oxidative stress. In one of the earlier experimental studies evaluating the association of varicoceles and oxidative stress, Weese *et al.* (1993) found the ROS concentrations to be higher in the semen samples of men with varicoceles, irrespective of their fertility status. A subsequent experimental study on rats confirmed that creation of an artificial varicocele bilaterally, could result in increased oxidative stress (Ozdamar *et al.*, 2004).

Varicoceles may exert their detrimental effects on spermatogenesis through changes in the hormonal milieu. Several studies have shown a decrease in the testosterone levels in men with varicoceles. This may be due to a time dependent decline in the testosterone of men with varicoceles, when compared with those without varicoceles. Other mechanisms for a decline in testosterone may be a poor responsiveness to hCG, decreased binding of hCG to Leydig cells, abnormality of peak secretions, or abnormalities of sex hormone binding globulin (Naughton *et al.*, 2001). However, there is no evidence to conclusively suggest the role of supplementation testosterone in the therapy of these patients, particularly since while the level of testosterone may be lower in men with varicoceles, it is often still within the normal range.

Other hormonal markers that have been studied include the Anti-Mullerian Hormone (AMH) and inhibin B. Both these hormones, which reflect poor germ cell/sertoli cell function, may be higher in prepubertal patients with a varicocele than in controls (Trigo *et al.*, 2004).

The effect of unilateral varicocele on ipsilateral testicular morphology and function has been well studied in human and experimental animals (Hendin *et al.*, 1999; Semercioz and Honor, 2003; Ozdamar *et al.*, 2004). The effect of unilateral varicocele on contralateral testicular function has not however been as extensively studied. Indeed the magnitude and extent of this so called sympathetic testicular damage remains unclear.

The aim of the present study therefore is to evaluate the effect of left experimental varicocele on the right epididymal sperm quantity, quality, fertilizing capacity and testicular histo-morphometry.

MATERIALS AND METHODS

Animals: Five-week-old immature male Sprague-Dawley rats weighing 100-130 g were used for the study. The animals were housed in wire mesh cages under standard environmental conditions with the provision of 12 h light and 12 h darkness. Rat cubes (Pfizer feeds Nig. Ltd., Lagos, Nigeria) and water were provided *ad libitum*.

Experimental protocol: Fifty five male rats were weighed and divided randomly into two groups. Group A (45 rats) served as the experimental group in which the rats were rendered unilateral varicocelized. To induce varicocele, the animals were anaesthetized with intra-abdominal injection of 7 mg kg^{-1} body weight ketamine hydrochloride. A 2 cm median incision was made through the skin, beginning caudal to the prepuce and extending cranially. The left spermatic vein was exposed and ligated completely with a 4-0 nylon suture as described by Sofikitis and Miyagawa (1992). Sixteen weeks after varicocele induction, all the rats were processed for the assessment of their fertility potential *in vivo*. Thereafter all the rats were sacrificed by decapitation. Testicular weight and volume, epididymal sperm characteristics and testicular histology were evaluated.

Fertility potential *in vivo*: Two fertile female Sprague-Dawley rats in the first hours of estrus as determined by vaginal smear examination were placed in a single cage with each male rat. Two hours later, the female rats were checked after mating to detect spermatozoa in their vagina by microscopic examination of the vaginal fluid. Females in which spermatozoa were detected were then checked 3 times daily from day 21 for parturition (day of mating taken as day 1). A male rat was considered fertile if its mating resulted in at least one pregnancy.

Organ weight and volume estimation: The testes were excised, dissected free of surrounding tissue, their weight determined and volume measured by water displacement method.

Sperm characteristics: The testes from each rat were carefully exposed and removed. They were trimmed free of the epididymides and adjoining tissues. From each separated epididymis, the cauda part was removed and placed in a beaker containing 1 mL physiological saline

solution. Each section was quickly macerated with a pair of sharp scissors and left for a few minutes to liberate its spermatozoa into the saline solution. Sperm motility, concentration and progressive motility were determined as earlier described (Raji *et al*., 2005, 2006). Semen drops were placed on the slide and two drops of warm 2.9% sodium citrate were added. The slide was covered with a cover slip and examined under the microscope using x40 objective for sperm motility. Sperm count was done under the microscope using improved Neubauer haemocytometer.

Histological analysis: The organs were cut in slabs of about 0.5 cm thick and fixed in Bouin's fluid for a day after which it was transferred to 70% alcohol for dehydration. The tissues were passed through 90% alcohol and chloroform for different durations before they were transferred into two changes of molten paraffin wax for 20 min each in an oven at 57°C. Prior to embedding, it was ensured that the sections to be cut by the microtome were orientated perpendicular to the long axis of the testis. Serial sections of 5 μm thick were obtained from a solid block of tissue and were stained with haematoxylin and eosin stains, after which they were passed through a mixture of equal concentration of xylene and alcohol. Following clearance in xylene, the tissues were oven-dried. Light microscopy was used for the evaluations.

Morphometric analysis: For each testis 5 sections from the polar and equatorial regions were sampled and an unbiased numerical estimation of the mean Seminiferous Tubular Diameter (STD) was done using a systemic random scheme (Gundersen and Jensen, 1987). The mean STD was derived by taking the average of two diameters d_1 and d_2. d_1 and d_2 are taken only when $d_1/d_2 \geq 0.85$.

Statistical analysis: Data are expressed as mean±standard error of the mean (M±SEM). The significance of difference was at $p<0.05$. Statistical analysis was performed using the student t-test and ANOVA.

RESULTS

Body weight changes: Table 1 shows that rats in control group had significant ($p<0.05$) increase in weight. Varicocelized rats lost weights when compared with their initial weights.

Weight and volumes of testes: Table 1 shows that left testicular weight and volume were significantly lower ($p<0.05$) than the right testicular weight and volume in varicocelized rats. The right testicular weights and volumes in varicocele rats were however also significantly lower ($p<0.05$) compared to the testicular weights and volumes of the control group.

Table 1: Body weight (g), Testicular weight (g) and volume (mL) and seminiferous tubules (ST) diameter (μm)

	Varicocelized (n = 45)		Control (n = 10)	
Parameters	Left	Right	Left	Right
Initial live body wt.	120.100±8.00		118.24±9.12	
Final live body wt.	115.720±5.12		135.06±7.20	
Body wt. difference	4.38 (3.65)		16.82 (14.22)	
Testicular weight	0.196±0.12*	0.86±0.10*	1.23±0.13	1.24±0.11
Testicular volume	0.180±0.14*	0.87±0.12*	1.22±0.14	1.23±0.10
ST diameter	110.350±3.47*	134.00±2.36*	185.64±3.63	188.67±4.50

Values are expressed as Mean±SEM, *$p<0.05$ compared to control group, ( ): Percentage

Table 2: Sperm characteristics and fertility rate

	Varicocelized (n = 45)		Control (n = 10)	
Parameters	Left	Right	Left	Right
Sperm conc. (10^6 mL^{-1})	15.80±4.30*	35.53±5.45*	78.54±3.30	74.50±4.35
Sperm motility (%)	7.52±2.10*	30.52±9.05*	85.42±6.75	72.53±1.84
Morphology % normal	62.35±1.30	73.50±2.40	95.56±6.52	94.06±5.50
% abnormal Ψ	37.65±2.45*	26.50±1.55*	4.44±1.56	5.94±2.46
Progressivity	b_1	b_1	a_1	a_1
Fertile	5 [15.4]*		9 [90]	

Values expressed as Mean±SEM, *$p<0.05$ compared with the control group, [ ]: Percentage, a_1: Rapid linear progressive motility, b_1: Sluggish linear or non-linear motility, Ψ in this study a spermatozoon was considered abnormal morphologically if it had one or more of the following features: rudimentary tail, round head and detailed head

Epididymal sperm parameters: As shown in Table 2, the sperm content and percentage motility were significantly lower ($p<0.05$) in the left epididymides than the right epididymides of the varicocele rats. However, the caudal epididymal sperm concentration and percentage sperm motility were significantly lower ($p<0.05$) bilaterally in the varicocele group compared to the control rats. Further, the percentage of morphologically abnormal spermatozoa was significantly higher ($p<0.05$) in both the left and right testes of the varicocelized animals when compared to the control group. The sperm cells from both caudal epididymides of the varicocele rats showed sluggish non linear movement while those from the control group demonstrated rapid linear motility.

Fertility potential *in vivo*: As shown in Table 2, the proportion of fertile rats in the varicocele group was significantly lower ($p<0.05$) than the control group.

Histo-morphometry: Light microscopy was used for evaluation of testicular histology as shown in Fig. 1-3. The seminiferous tubules of the control rats were completely and fully differentiated. Spermatozoa are

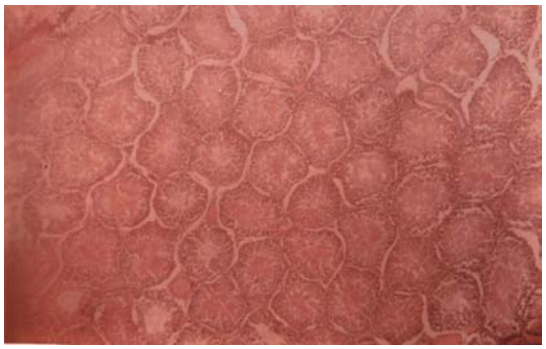

Fig. 1: Section through testis of control rats H and E (x40)

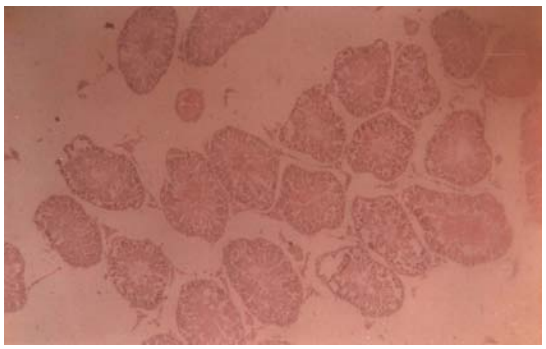

Fig. 2: Section through left testis of varicocelized rats H and E (x40)

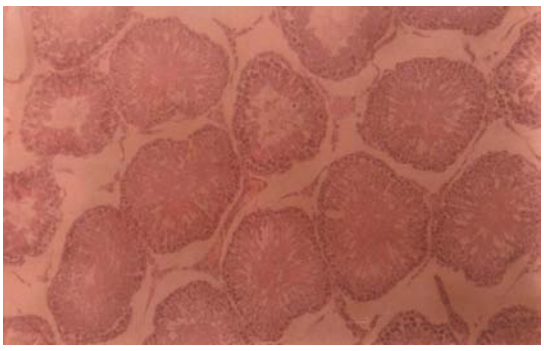

Fig. 3: Section through right testis of varicocelized rats H and E (x40)

shown in some of the tubules. However, in the varicocele group, the testis showed degeneration of the seminiferous tubules and vacuolation of the stroma. These degenerative features were more prominent in the left varicocelized testis than in the right testis where varicocele was not induced. The mean seminiferous tubular diameters of bilateral testes in varicocele rats were significantly lower ($p<0.05$) than those of the control group. In the varicocele group, the left varicocelized testes mean seminiferous diameter was significantly lower ($p<0.05$) compared to that of the right testes (Table 1).

DISCUSSION

Varicocele is formed of dilated veins in the pampiniform plexus of the spermatic cord. In general, a prevalence proportion of 15-20% is assumed in the male population and in approximately 30-40% of men presenting with infertility (Jarow, 2001). Although varicocele is a common problem in adulthood, it is rarely detected in prepubertal boys with an incidence rate of 2 to 11% (Kubal *et al*., 2004). The prevalence of varicoceles in pubertal men is comparable to that in adult population and suggests that physiological changes associated with puberty, such as increase in testicular mass and in testicular blood flow, may play some role in varicocele formation (Sawczuk *et al*., 1993). Varicocele, which a leading cause of male infertility is associated with increased production of spermatozoal ROS. Irrespective of the infertility status, it was identified that there is a strong relationship between sperm dysfunction and varicocele (Lewis *et al*., 1995). Indeed Hendin *et al*. (1999) reported a 4-fold increase in the frequency of elevated ROS generation in the incidental varicocele group compared to their control patients.

For anatomical reasons varicoceles occur more commonly on the left side. Bilateral varicoceles are present less frequently. Right-sided-only varicoceles are rare. In this study a left sided varicocele was experimentally induced in adolescent male Sprague Dawley rats. Clinical studies in adolescents had shown that varicocele early in puberty will often lead to testicular volume loss (Kass *et al*., 2001). Choi *et al*. (1990) indicated that experimental varicocele in adolescent rats led to more testicular abnormality than those in adult rats. The results from the present study showed a derangement of the growth and reproductive functions of the animals. The gain in live body weight of the control rats meant that the rats were still in the active growth phase. The loss in live body weight of the experimental group indicates that artificial varicocele has a negative effect on the body metabolic process (Ozdamar *et al*., 2004). The procedure for achieving artificial varicocele confers a conspicuous level of stress on the rats. This stress could affect their metabolic process leading to losses in live weights observed in this study.

The significantly lower weights of the left testes in varicocelized group compared to the control corroborate the report of Suziki and Sofikitis (1999). Semercioz and Honor (2003), also reported degenerative changes in the

seminiferous epithelium of testes that were made varicocelized, due to the effects of heat from venous stasis on the spermatogenic cells within the epithelium leading to loss in testicular weight. Present study however demonstrated that unilateral varicocele induced in the immature rats before sexual maturation resulted in a significant bilateral impairment of testicular functions. The significant bilateral decrease in the mean testicular weight and volume in the varicocelized rats indicates bilateral testicular dysfunction. This is because testicular size has a positive correlation with testicular function.

Earlier experimental studies have suggested that mean seminiferous tubular diameter is a more sensitive early indicator of contralateral testicular deterioration (Karaguzel *et al*., 1995; Zhang *et al*., 2002). Present study with data showing significant reduction in the mean seminiferous tubular diameters in varicocelized rats therefore corroborates these findings.

A detrimental effect of unilateral testicular function is additionally indicated by the significant reduction of the quantitative and qualitative sperm parameters bilaterally in animals with left experimental varicocele. Logical fallout of these poor sperm characteristics in both the left and right testes of the varicocelized group is the significant reduction in the fertility potential *in vivo* observed in this group.

The ipsilateral testicular dysfunction that occurs with unilateral varicocele may be explained by increase in ipsilateral testicular temperature leading to oxidative stress. However, there is much controversy concerning the mechanisms by which unilateral varicocele produces contralateral testicular toxicity. A plausible hypothesis could be that contralateral testicular deterioration may result from a reflex mechanism probably as in conditions such as consensual opthalmic reflex and reflex anuria. The possibility of this so called sympathetic injury in respect of the testis was shown in the earlier study (Saalu *et al*., 2007). Similarly, Stokes *et al*. (1988) demonstrated the bilateral effects of unilateral vasectomy in rats.

In conclusion, this study demonstrated a detrimental effect of left varicocele on the right testicular function. Further studies are however required to investigate the progressitivity of these findings. This is so because progressive deterioration of bilateral testicular function in unilateral varicocele will support the clamour for early varicocelectomy to protect both the ipsilateral and contralateral testes.

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