Increased expression of interleukin-1α and interleukin-1β is associated with experimental varicocele

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Objective: To describe the effect of varicocele, in an experimental rat model, on the levels of IL-1α and IL-1β proteins in testis tissue.
Design: Comparative and controlled study.
Setting: Experimental research.
Animal(s): Wistar male rats in experimental and control groups.
Intervention(s): The control group underwent sham operation (n = 6). Experimental groups underwent partial ligation of the renal vein to induce experimental varicocele and were then killed at 9 (n = 6), 11 (n = 6), and 13 (n = 6) weeks after induction of varicocele.
Main Outcome Measure(s): Histologic evaluation of the varicocele model was determined by periodic acid-Schiff staining of paraffin-embeded testicular tissues. Levels of cytokines were assessed by immunohistochemistry and Western blot analysis.
Result(s): Varicocele caused testicular damage, especially in 11- and 13-week-old varicocele groups. In sham-operated rats, Golgi complexes of round spermatids expressed especially the α form of IL-1. By the progression of varicocele, the IL-1α expression increased temporally in Sertoli cells, spermatogonia, primary spermatocytes, spermatids, and Leydig cells. The expression of IL-1β was seen in Leydig cells in sham-operated rats. The IL-1β expression was also increased upon progression of varicocele in Leydig cells, Sertoli cells, and spermatogonia.
Conclusion(s): We suggest that IL-1α and IL-1β are the regulators of testicular function. Certain pathologic conditions, e.g., varicocele, cause an increase in the expressions of such proinflammatory cytokines. The increased expression of IL-1α and IL-1β in varicocele shifts the balance in favor of inflammatory and immune responses and causes detrimental effects in testis tissue, which may cause male infertility. (Fertil Steril® 2006; 85(Suppl 1):1265–75. ©2006 by American Society for Reproductive Medicine.)

Key Words: IL-1α, IL-1β, experimental varicocele, rat, testis

Varicocele is the most common etiologic factor associated with male infertility. It is a vascular abnormality of the scrotum that is defined as dilated veins of the pampiniform plexus. There is significant evidence in the literature that varicocele has an adverse effect upon the testis and its function (1). The pathophysiologies of the testicular damage in varicocele are not completely understood; however, gross testicular alterations associated with varicocele are well documented. Several studies have been carried out to give explanation for the altered spermatogenesis associated with varicocele (2, 3). Despite much conjecture, there is no conclusive data favoring any of these hypotheses.

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Cytokines are small soluble protein molecules that are important in the regulation of inflammatory responses as well as in the generation of immunity to pathogens. Also, they transmit information from one cell to another and regulate cell growth and differentiation. They all bind to specific receptors expressed on the surface of the target cell. They thereby trigger complex intracellular signaling events, which control gene expression required for the cellular response. These mediators usually have an effect on closely adjacent cells, and therefore function in a predominantly paracrine fashion. They may also act at a distance (endocrine), and they may have effects on the cell of origin (autocrine) (4).

Interleukin-1 (IL-1) is a member of the cytokine family having several important biologic activities, such as the control of cell proliferation, induction of fever, and stimulation of production of other cytokines. Interleukin-1 exists in two forms, the acidic form, IL-1α, and the neutral form, IL-1β, and is synthesized by a wide variety of cells (5). They are coded by two different genes but bind to the same

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receptor (6). Molecular studies have demonstrated that two types of IL-1 are produced as 31-kDa precursor and secreted as 17 kDa, and both precursor and mature form of the protein are capable of nuclear binding and may act as an active protein (7). However, the testis shows distinct molecular protein species which are IL-1–like factors with apparent molecular sizes of 45, 24, and 19 kDa rather than the known 17-kDa peptide or its 31-kDa precursor (7, 8).

In rat and mouse testicular interstitial tissue, Leydig cells and macrophages secrete IL-1, predominantly the β form, whereas within the seminiferous tubules, Sertoli cells and possibly germ cells produce the α form of IL-1. Sertoli cell IL-1 secretion increases during sexual maturation and fluctuates during the seminiferous epithelium cycle (9).

Different physiologic and pathologic factors affect the IL-1 levels in the testis (10). Testicular IL-1 messenger RNA (mRNA) and protein can be regulated by gonadotropin hormones and by known activators of macrophage/monocyte IL-1, such as lipopolysaccharides and latex beads (11). It is known that the increased expression of these proinflammatory cytokines results in testicular dysfunction (12). For example, after inflammation, the up-regulation of IL-1 in circulation or the testis undoubtedly induces the negative role of this cytokine, which is important in the regulation of spermatogenetic cycles. Therefore, it is believed that the extreme expression of this cytokine in the testis because of disease or injury may be the reason for disordered spermatogenesis and steroidogenesis (13).

As mentioned above, IL-1α and IL-1β are suggested to play a regulatory role in testicular function and thus may be critical in fertility potential. Although there are a lot of histopathologic studies on human varicocele and its negative effects on fertility potential (1), to the best of our knowledge there is only one study which determined the levels of IL-1 in testis tissue with varicocele (14). However, the cellular distribution of IL-1α and IL-1β in testis tissue and how the varicocele pathology affects these protein expressions remain to be elucidated.

In the present study we aimed to clarify the temporal expression patterns of these cytokines in rat testicular tissue, and thus their contribution to possible male infertility caused by varicocele is discussed.

MATERIALS AND METHODS

Animals

A total of 24 male *Rattus norvegicus* (Wistar) rats were allowed free access to standard rat laboratory diet and tap water. Adult animals (18 weeks old) were used for the control group and underwent a sham operation (n = 6). Seven-week-old 160–170-g animals underwent partial ligation of the renal vein (n = 18) and were used for the experimental groups. A minimum of six animals were sampled at each of the following time points after creation of varicocele: 9 weeks, 11 weeks, 13 weeks.

After anesthesia using xylasine (15 mg/kg IM Rompun; Bayer AG, Leverkusen, Germany) and ketamine (75 mg/kg IM Ketalar; Parke-Davis, Istanbul, Turkey), the experimental varicocele was created in 18 rats as previously described (2).

The upper left abdominal quadrant was approached through a midline laparotomy incision. The abdominal contents were packed to the right in order to visualize the left kidney, adrenal, and renal veins and the left spermatic vein as it inserts into the left renal vein. Using careful blunt dissection, a tunnel was made in the fat and connective tissues surrounding the left renal vein, and the vein was cleared of adhering tissue in a position medial to the insertion of the spermatic and adrenal veins. A 4-0 silk suture was tied to partially occlude the left renal vein at the point the vessel was cleared of other tissue. The ligature was made around a metal wire of 0.85 mm diameter. The wire was removed from the ligature, and the vessel expanded to an external diameter of approximately 1 mm.

After instillation of 10 mg/mL gentamicin sulphate into the abdominal cavity, the midline incisions of abdominal wall and anterior abdominal muscles were separately repaired using 4-0 chromic catgut. After identification of left varicocele by observing the dilatation of the internal spermatic veins (2), both testicles were then harvested from the same incision. All surgical procedures were performed under 3.5× magnification. Sham-operated rats underwent a similar procedure: The left renal vein was dissected free but not ligated.

We performed a midline abdominal incision and explored the left spermatic vein to measure its diameter with a metal micrometer to determine the varicocele. All experimental group rats that failed to form a left varicocele of $<$1 mm left spermatic vein diameter were excluded from the study.

The experimental protocol was approved by the animal core and usage committee of Akdeniz University and was in accordance with Declaration of Helsinki and International Association for the Study of Pain guidelines.

Tissue Processing

Testes were obtained from sham-operated rats as a control and varicocele-induced adult Wistar rats weighing 200–250 g. Animals were killed at 9, 11, and 13 weeks after creation of varicocele. Dilatation of the internal spermatic veins was identified during the killing procedure. Both testes were delivered into the abdomen. After harvesting the left testes, they were divided into two pieces: One piece was routinely processed for immunohistochemistry and the other piece was homogenized for Western blotting.

Histologic evaluation of our varicocele model was carried out by periodic acid-Schiff (PAS) staining by comparing

TABLE 1

Interleukin-1α and IL-1β immunoreactivity in sham-operated and varicocele-induced rats.

	Sham		9-week varicocele		11-week varicocele		13-week varicocele	
Cell type	IL-1α	IL-1β	IL-1α	IL-1β	IL-1α	IL-1β	IL-1α	IL-1β
Leydig	+	+	++	++	++	+++	+++	+++
Sertoli	+	0	+	+	++	++	+++	+++
Spermatogonia	0	0	+	0	++	+	+++	++
Primary spermatocyte	+	0	++	0	+++	0	+++	0
Spermatid								
Round	++	0	++	0	++	0	+++	0
Elongate	++	0	++	0	++	0	+++	0
Sperm	0	0	0	0	+	0	++	0

0: Negative; +: weak positive; ++: positive; +++: dense positive.

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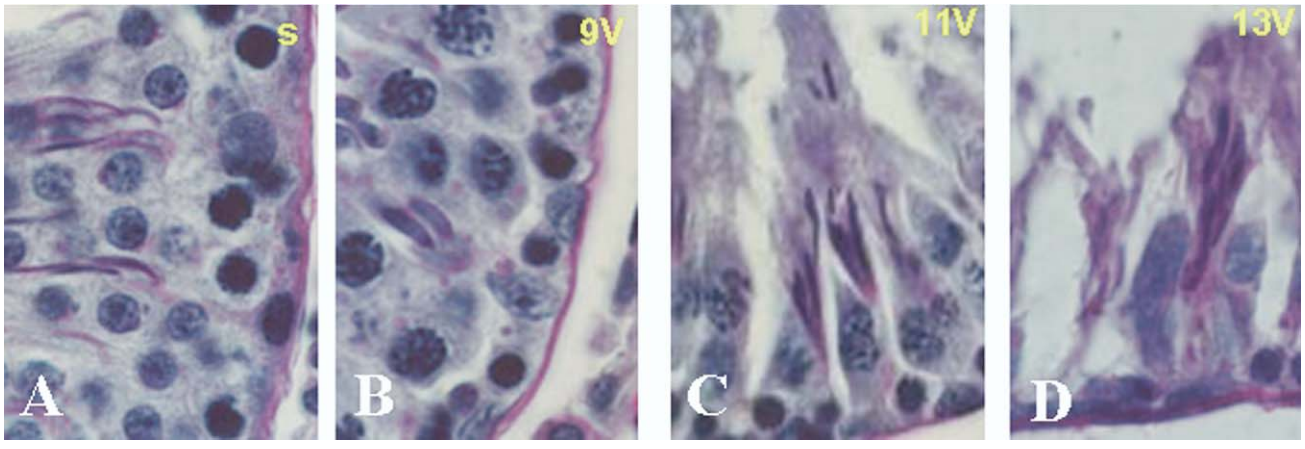
sham and experimental groups, in which disintegration of the germinal epithelium and degenerative appearance were easily identified.

Immunohistochemistry

For immunohistochemical assays, Bouin-fixed sections were deparaffinized and blocked for endogenous peroxidase activity with methanol containing 3% H_2O_2 for 10 min and for nonspecific binding with universal blocking reagent (BioGenex, San Ramon, CA) for 10 min at room temperature. Rabbit polyclonal IL-1α (sc-7929 (H-159); Santa Cruz, Santa Cruz, CA) and rabbit polyclonal IL-1β (sc-7884 (H-153); Santa Cruz) primary antibodies were applied as 1:400 and 1:200 dilutions (0.5 and 1 μg IgG/mL), respectively, overnight at +4°C. Negative control staining was performed by replacing the primary antibodies with their nonimmune isotypes at the same concentrations. The signals were developed by an avidin–biotin–horseradish peroxidase complex using Universal LSAB kit (Dako, Glostrup, Denmark). Antibody complexes were visualized by incubation with diaminobenzidine chromogen (BioGenex). Sections were counterstained with Mayer's hematoxylin (Dako), for 30 s, dehydrated, mounted, and examined by light microscopy.

FIGURE 1

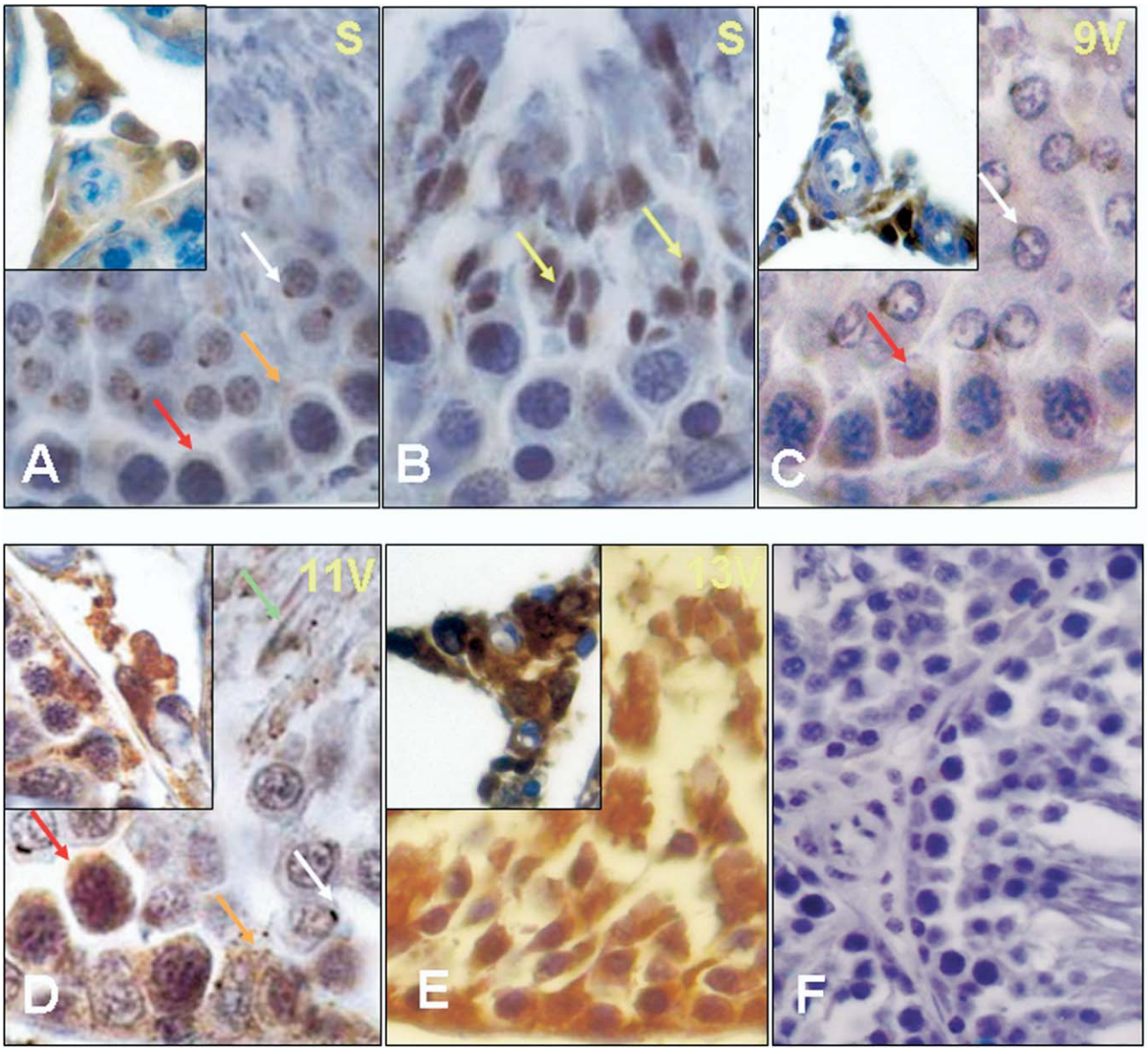
Histochemical Periodic Acid Schiff (PAS) staining of paraffin-embeded rat testicular tissues. When compared to the control sham operated rats (**A**), 9-week (**B**), 11-week (**C**), and 13-week (**D**) varicocele-induced rats showed an apparent degeneration of the tubules related to the progressing weeks of varicocele. Magnification 100×.



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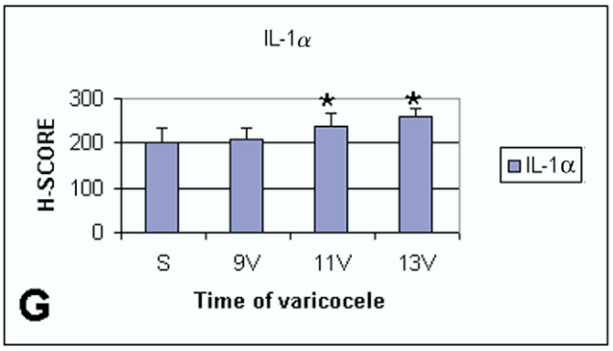
FIGURE 2

Interleukin-1α immunostaining in sham group (**A, B**) and varicocele-induced groups (**C–E**). Magnification 100×. (**A, B**) In the sham group (S), immunostaining was dense in Golgi complexes of round spermatids (*white arrow*) and in heads of elongated spermatids (*yellow arrow*) but weak in primary spermatocytes (*red arrow*), in Sertoli cells (*orange arrow*), and in Leydig cells (**inset**). (**C**) In the 9-week varicocele group (9V), immunostaining was similar in Golgi complexes of round spermatids (*white arrow*) but slightly increased in primary spermatocytes (*red arrow*) and in Leydig cells (**inset**) compared to S. (**D**) In the 11-week varicocele group (11V), expression was similar in Golgi complexes of round spermatids (*white arrow*) but increased in primary spermatocytes (*red arrow*), in Sertoli cells (*orange arrow*), and in Leydig cells (**inset**) compared to S. Sperm cells also were positive (*green arrow*). (**E**) In the 13-week varicocele group (13V), immunostaining increased densely in all cell types of seminiferous tubuli compared to S. (**F**) Negative control section. (**G**) H-SCORE analysis of immunostaining in sham and varicocele-induced groups. *$P<.05$.



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FIGURE 2 CONTINUED



Sahin. IL-1α and IL-1β expressions in varicocele. Fertil Steril 2006.

SDS-PAGE and Western Blotting

Protein extraction and immunoblot analysis were performed as previously described (15). Tissues were weighed and put into homogenation buffer (10 mmol/L Tris-HCL, 1 mmol/L EDTA, 2.5% sodium dodecyl sulfate (SDS), 1 mmol/L phenylmethylsulfonylfluroide, and 1μg/mL leupeptin) supplemented with protease inhibitor cocktail (Complete; Boehringer, Mannheim, Germany). After being homogenized, samples were centrifuged at 10,000*g* for 10 min. Supernatants were collected and stored at −70°C.

The protein concentration was determined by Lowry assay (16), and 50 μg protein was applied per lane. Prior to electrophoresis, samples were boiled for 5 min at 95°C. Samples were subjected to SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and then were transfered onto nitrocellulose membranes (Amersham Pharmacia, Piscataway, NJ) in a buffer containing 0.2 mol/L glycine, 25 mmol/L Tris, and 20% methanol overnight. Successful transfer was confirmed by Ponceau S (Sigma) staining of the blots. The membranes were blocked for 2 h with 5% nonfat dry milk (BioRad, Hercules, CA) and 0.1% Tween-20 (Sigma) in 0.14 mol/L Tris-buffered saline (TBS), pH 7.2–7.4, at 4°C. Blotting membranes were incubated for 2 h at room temperature with 1:100 dilution of IL-1α (sc-7929 (H-159), Santa Cruz) and IL-1β (sc-7884 (H-153), Santa Cruz). After washing steps, the membranes were further incubated with goat antirabbit IgG horseradish peroxidase conjugate (BioRad) diluted 1:5,000 for 1 h at room temperature.

Immunolabeling was visualized using the chemiluminescence-based Super Signal CL-HRP Substrate System (Pierce, Rockford, IL) and the membranes were exposed to Hyperfilm (Amersham), which was subsequently quantified using an Alpha Digi Doc 1000 gel documentation unit (Alpha Innotech Corporation, San Leandro, CA). Control blots were incubated with antibody diluent only instead of the primary antiserum. β-Actin antibody (1:5,000 dilution) was used as an internal control for each blotting in order to confirm the equal loading of the samples.

H-SCORE and Semiquantitative Evaluations

The evaluations of the immunohistochemical staining of both sham and varicocele groups were done utilizing H-SCORE (2). Briefly, stained sections were evaluated using an Axioplan microscope (Zeiss, Oberkochen, Germany) with a special ocular scale. From each three randomly selected slides, five different fields at 200× magnification were evaluated for the analysis of immunohistochemical staining of the antibodies. The staining was scored in a semiquantitative fashion that included the intensity of specific staining in sections. The evaluations were recorded as percentages of positive-stained cells of all type for both proteins of four intensity categories: 0 (none), 1+ (weak but detectable above control), 2+ (distinct), 3+ (intense). For each tissue, an H-SCORE value was derived by summing the percentages of cells that stained at each intensity multiplied by the weighted intensity of the staining (H-SCORE = $S P_i (i + 1)$, where i is the intensity score and P_i is the corresponding percentage of the cells). The H-SCORE values were graphed.

Two observers blinded to the experimental groups performed the H-SCORE, and interindividual variation was 8%. The distributions of positively immunoreactive cells in all experimental groups were also determined semiquantitatively (0: negative; +: positive; ++: dense positive; +++: very dense positive) and presented in Table 1.

Statistical Analysis

Statistical analyses of immunohistochemical quantifications were compared by Mann-Whitney U test. Statistical analyses of IL-1α and IL-1β Western blot quantifications were evaluated by one-way ANOVA tests followed by post hoc Tukey tests for parametric data. Comparisons were made versus the sham-operated group as a control. Probability values of less than .05 were considered significant; values are presented as mean ± SEM.

RESULTS

Histologic Evaluation of the Varicocele Model

Histologic evaluation of the experimental model was determined in testis tissue specimens harvested from sham and 9, 11, and 13 weeks of varicocele groups with PAS staining. Disintegration of the germinal epithelium and degenerative appearance, especially in 11- and 13-week varicocele groups, was observed visibly when compared to sham group by light microscopy (Fig. 1).

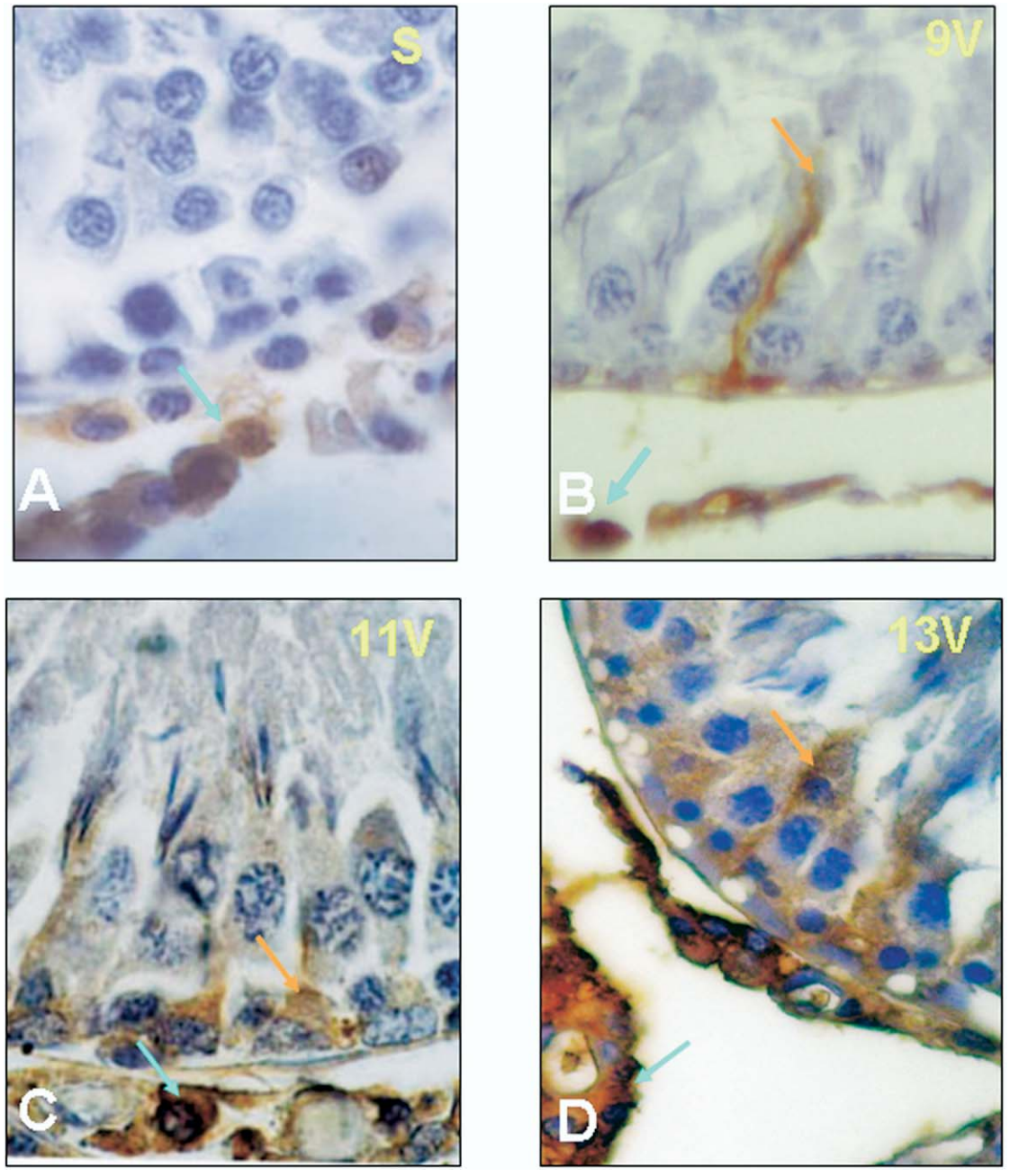
Immunohistochemical Results

Semiquantitative immunolabeling density distribution patterns of IL-1α and IL-1β in sham-operated and varicocele groups of testis tissues are shown in Table 1.

IL-1α. In sham-operated rats, IL-1α immunostaining was observed densely in Golgi complexes of round spermatids (Fig. 2A) and in heads of elongated spermatids (Fig. 2B).

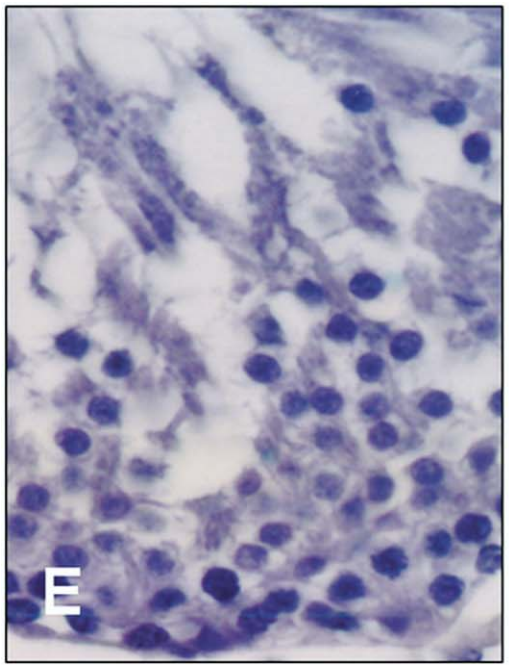
FIGURE 3

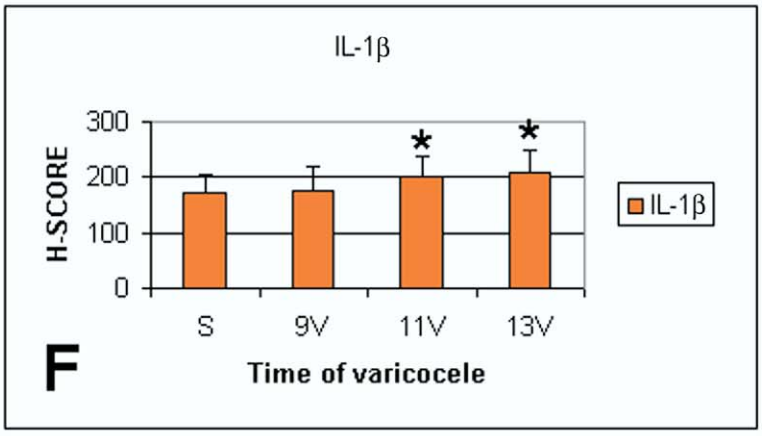
Interleukin-1β immunostaining in sham group (**A**) and varicocele-induced groups (**B–D**). Magnification 100×. In S (**A**), Leydig cells (*green arrow*) were positive. In 9V (**B**), 11V (**C**), and 13V (**D**), the density of immunostaining increased in Leydig cells (*green arrow*) and in Sertoli cells (*orange arrow*) with the progression of varicocele. (**E**) Negative control section. (**F**) H-SCORE analysis of immunostaining in sham and varicocele-induced groups. *$P<.05$. Abbreviations as in Figure 2.



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FIGURE 3 CONTINUED





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Additionally, primary spermatocytes and Sertoli cells (Fig. 2A) had weak positive immunoreactivity, whereas Leydig cells (Fig. 2A, inset) had positive immunoreactivity for IL-1α. In sham-operated rats, there was no positive immunoreactivity of IL-1α in spermatogonia and in sperm cells (Fig. 2A and B).

In the 9-week varicocele group, IL-1α expression was present in Golgi complexes of round spermatids (Fig. 2C) similar to the sham-operated group, while its expression in spermatogonia, in primary spermatocytes (Fig. 2C), and in Leydig cells (Fig. 2C, inset) slightly increased compared to the sham-operated group. There was no immunoreactivity in sperm cells.

In the 11-week varicocele group, although the expression of IL-1α was similar to the sham-operated group in Golgi complexes of round spermatids, the expression of this protein increased in spermatogonia, in primary spermatocytes, and in Sertoli and Leydig cells along with the induction of varicocele (Fig. 2D, inset). Additionally, sperm cells were also positive for IL-1α protein in this group (Fig. 2D).

Finally, in the 13-week varicocele group, the immunoreactivity increased densely in all cell types of seminiferous tubuli (Fig. 2E and inset) compared to the sham-operated rats. Negative control incubations utilizing normal rabbit serum confirmed the specificity of the labeling (Fig. 2F).

The H-SCORE analysis revealed that there was no significant difference in immunostaining densities of IL-1α between sham-operated rats and the 9-week varicocele group; whereas comparisons of the 11- and 13-week varicocele groups with the sham group showed that the expression of IL-1α increased significantly ($P<.05$) (Fig. 2G).

IL-1β. In sham-operated rats, positive IL-1β immunostaining was present especially in Leydig cells (Fig. 3A).

In the 9-week varicocele group, the immunostaining intensity of Leydig cells increased compared to sham (Fig. 3B). Furthermore, in this experimental group, there was a positive immunostaining in Sertoli cells (Fig. 3B) which was not observed in sham-operated rats.

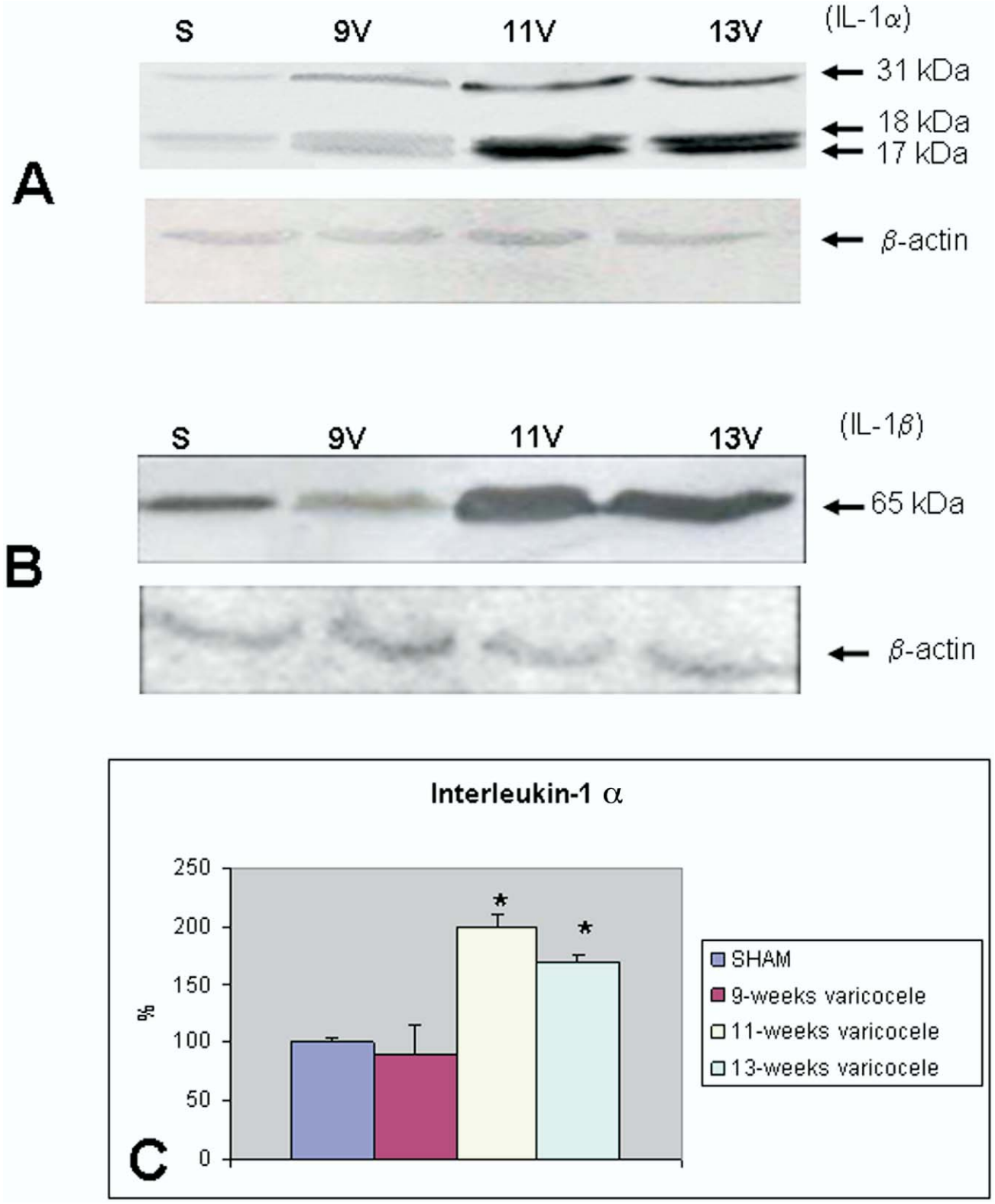
In the 11-week varicocele group, the density of immunostaining was increased in Leydig cells, in Sertoli cells, and in spermatogonia with the progressing varicocele week compared to the sham and 9-week varicocele groups (Fig. 3C).

In the 13-week varicocele group, immunostaining continued to increase in Leydig cells, in Sertoli cells, and in spermatogonia (Fig. 3D). Sperm cells had no immunopositivity for IL-1β in all groups that were studied. Specificity of the IL-1β labeling was tested by using normal rabbit serum at the same concentration with the protein (Fig. 3E).

The H-SCORE analysis revealed that there was no significant difference between the sham-operated and 9-week varicocele groups; whereas comparisons of the 11- and 13-week varicocele groups with the sham group showed that the expression of IL-1β was increased significantly ($P<.05$) (Fig. 3F).

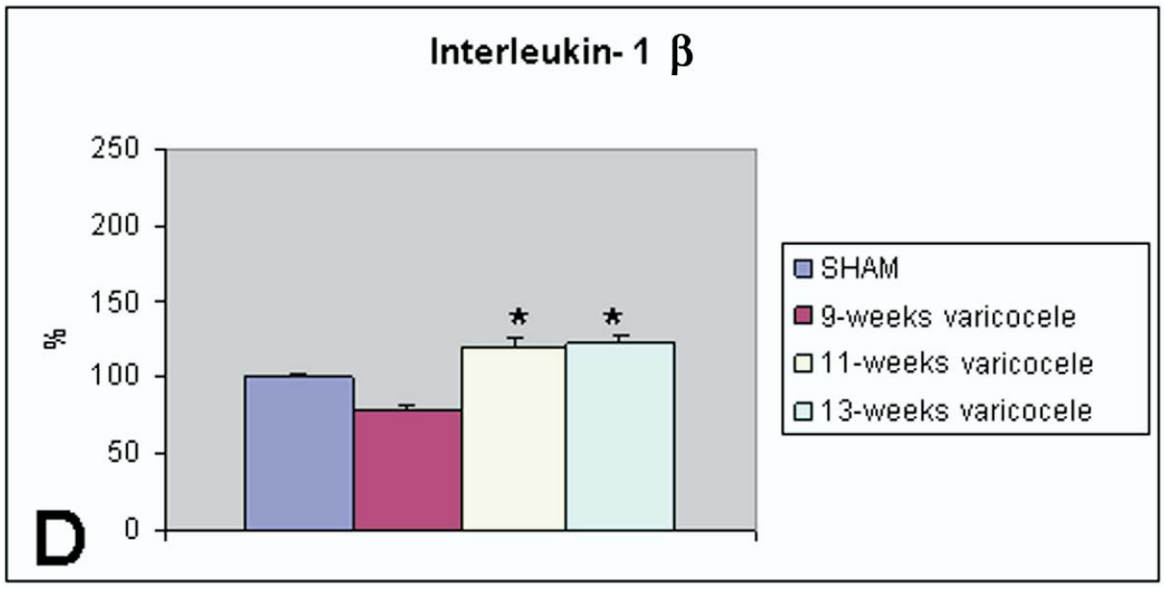
FIGURE 4

Western blot results of IL-1α (**A**) and IL-1β (**B**) proteins in sham and varicocele-induced groups. β-Actin was used as an internal standard. Quantitative analysis of IL-1α (**C**) and IL-1β (**D**) Western blot data. The values in the bar graphs were obtained by dividing the protein intensity value to its corresponding β-actin intensity value. *$P<.05$. Abbreviations as Figure 2.



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FIGURE 4 CONTINUED



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Western Blot

As depicted in Figure 4A and B, lysates of testis from the sham-operated and varicocele-induced groups expressed IL-1α (17 and 31 kDa) and IL-1α–like molecule (18 kDa) as well as IL-1β–like molecule (65 kDa). Because the IL-1 molecules detected were characterized by hitherto undescribed 18-kDa and 65-kDa components rather than by the known 17-kDa peptide or its 31-kDa precursor, we have chosen to use the term "IL-1–like molecule."

The protein levels of IL-1α and IL-1α–like and IL-1β-like molecules were significantly higher in the 11- and 13-week varicocele-induced groups ($P<.05$) than the levels in the sham-operated and 9-week varicocele groups (Fig. 4C and D). In all examined samples, IL-1–like molecules were characterized by the presence of a high-molecular-weight peptide (18 kDa for IL-1α and 65 kDa for IL-1β). In all samples of testicular cells from the sham-operated and varicocele-induced groups, we also observed the expression of 17 kDa and 31 kDa for IL-1α, as suggested by the manufacturer.

β-Actin antibody (1:5000 dilution) was used as an internal control for each blotting in order to confirm equal loading of the samples.

DISCUSSION

The present work demonstrates an experimental study which shows the effect of varicocele on the expression of IL-1α and IL-1β in rat testis tissue during the progressive course of such pathology. This study extends earlier studies that were performed on animals (17) and humans (18) and have suggested that varicocele produces a progressive duration-dependent detrimental effect on the testis tissue. However, the mechanism of testicular damage with varicocele and varicocele-related infertility remains unknown. In the present study, the detrimental effects caused by varicocele were discussed via IL-1α and IL-1β expression and give a new sight in varicocele-associated infertility and testis damage interaction.

Interleukin-1 is a proinflammatory cytokine that activates immunologic responses and inflammation (19). Interleukin-1 has been shown to affect both germ cell and immature Leydig cell proliferation (20) and to modulate adult Leydig and Sertoli cell function (21). Those experimental models support the concept that IL-1 plays a role in the autocrine and paracrine regulation of normal and pathophysiologic testicular function.

Some studies suggest a relationship between cytokine levels in seminal plasma and subfertility. Dousset et al. (22) measured the concentrations of interleukins (IL-1β, IL-2, IL-6) and those of interleukin soluble receptors (SR IL-2, SR IL-6) in semen of 21 fertile subjects and 119 patients with a range of andrologic disease. An increase of IL-1β was observed in the group of patients with infertility. Also Buch et al. (12) suggests that high levels of IL-1α have a detrimental effect on testicular physiology.

In this study, it is well established that in normal testis tissue Golgi complexes of round spermatids and elongated spermatids expressed especially the α form of IL-1. This may indicate a potent regulatory role for IL-1α in the maturation of spermatids in normal spermatogenetic process. Moreover, the IL-1α expression was up-regulated in sper-

matogonia, primary spermatocytes, spermatids, Leydig cells, and Sertoli cells with the progressing varicocele. These results indicate that varicocele, a potent proinflammatory activator, induces the up-regulation of IL-1α in different cell types, which may contribute to the ongoing survival of the highly immunogenic germ cells in testis tissue.

In the present study, in contrast to IL-1α, IL-1β expression was observed in Leydig cells in the sham-operated group. However, the IL-1β protein expression increased with the progression of varicocele in Leydig cells, Sertoli cells, and spermatogonia. The major physiologic role of Leydig cells is to produce androgen required for spermatogenesis. Numerous studies have suggested an important role for IL-1 in the regulation of Leydig cell steroidogenesis (23). However, while some studies have demonstrated an inhibitory effect of this cytokine in testosterone biosynthesis both in vitro and in vivo (24), others have described stimulatory actions of IL-1 on testosterone output by Leydig cells (25). The apparent discrepancies in the results may come from differences in the experimental conditions or methodologies.

Our study indicates that IL-1β expression may be related to Leydig cell function rather than germ cell maturation in normal in testis tissue. As suggested by other studies, IL-1α and IL-1β stimulate their own as well as each other's production in an amplification loop (26). In this study, the increased expression of IL-1β with varicocele pathology may result from the overexpression of IL-1α or of itself in a paracrine manner. The overexpression of IL-1β in Leydig cells, Sertoli cells, and spermatogonia, which was not seen in normal conditions, may alter the testosterone release and shifts steroidogenesis in inflamed testis tissue, damaging the spermatogenetic process.

These observations were further validated by the PAGE/Western blot analyses under denaturating conditions, showing that the amounts of proteins are increased by progressing varicocele. In activated macrophages, the expected IL-1 species are the 31-kDa precursor protein and the secreted mature 17-kDa form (7). In recent years, an IL-1–like activity was identified in the testis, seminiferous tubule–conditioned medium, and adult rat testis interstitial fluid (8, 9), originating from an alternate splicing of the pro-IL-1 primary transcript (27). In the present study, we observed 17- and 31-kDa molecular bands which are specific for IL-1α (7) and high-molecular-weight bands for IL-1α (18 kDa) and IL-1β (65 kDa), which are original results. These results show that both normal-molecular-weight bands and high-molecular-weight IL-1–like bands are peptides peculiar to testis tissue.

Many questions and mechanisms still remain to be elucidated to understand varicocele-related infertility. Data from the literature confirm that IL-1 takes part in spermatogenesis and steroidogenesis actively and that it is a regulators of testicular cell function. In the present study, IL-1–like activity in different molecular sizes not previously known was shown by Western blot analysis.

In conclusion, we believe that the observed histologic damages associated with varicocele are time dependent and may be the result of overexpression of these cytokines, which may lead to shifts the balance in favor of inflammatory and immune responses. However, the mechanism of the alterations requires further study.

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