ESTROGEN-TESTOSTERONE RATIO IN PLEXUS PAMPINIFORM

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ABSTRACT

Objective: To evaluate estrogen-testosterone ratio in rabbits with a left artificial varicocele. Material & Method: Eight rabbits were divided into two groups. The first group underwent ligation of left renal vein to induce artificial varicocele and the second group underwent a sham operation as control group. Eight weeks after ligation, blood samples were taken from the pampiniformplexus in both groups. Serologic examination (ELISA) was performed to measure testosterone and estrogen level. The t-test was used to compare the differences of the estrogen-testosterone ratios, a p value < 0.05 is considered significant. Results: Testosterone level was increased with 279% and estrogen level was increased with 33% blood from the pampiniform plexus in group 1 compared with group 2 (normal rabbits). The ratio of estrogen-testosterone between the control group and the artificial varicocele group were 1 : 3. Differences of the ratio between two groups were statistically significant (p < 0.05). Conclusion: Estrogen-testosterone ratio was lower in varicocele rabbit compared with normal rabbits.

Keywords: Estrogen-testosteron ratio, left artificial varicocele.

INTRODUCTION

Varicocele is a known cause of male infertility. About 20-40% of infertile men suffer varicocele. Spermatogenesis is regulated by a hormonal system through a complex mechanism. Testosterone is produced by Leydig cells that initiates and maintain spermatogenesis process. Testosterone regulate spermatogenesis by stimulation of Sertoli cells. A deficiency in testosterone hormone causes the cease of spermatogenesis process in the mitosis division phase of germinall cell. In testis tissues, particularly in Leydig cells, Sertoli cells, and germinal cells, aromatase enzyme and receptor estrogen can be found. Aromatase enzyme converts...
androgen into estrogen. The role of estrogen in spermatogenesis process is still controversial and under investigation. Spermatogenesis process is influenced by some reproductive hormones. To ensure that a spermatogenesis process is going on well a hormonal balance is required.

Theoretically, in varicocele there is static blood flow in pampiniform plexus and hypoxia that causes dysfunction of Leydig cells and disorder in the conversion of testosterone to estrogen.

This research was to determine whether in testis with varicocele there occur a change in the balance between testosterone and estrogen hormones, as a scientific proof in explaining the mechanism of spermatogenesis disorder occurrence in testis with varicocele.

**OBJECTIVE**

To determine whether there is a change in estrogen-testosterone ratio in the testis pampiniform plexus of rabbits with left artificial varicocele.

**MATERIAL & METHOD**

This research was an experimental study to determine any change in estrogen-testosterone ratio in the testis of rabbits with varicocele. It was conducted at Clinical Pathology Laboratory of Hasan Sadikin Hospital, in January-July 2010. The subjects of research included eight male rabbits, 1250-2500 gr in weight, 13-17 weeks old.

The eight rabbits were divided into two groups (A and B), each consisting of 4 rabbits. In the beginning of research the rabbits were weighted by a Lion Star spring, and measurement was performed by one researcher. An acclimation was made for 7 days. Group A underwent left renal vein ligation to induce artificial varicocele and group B underwent sham procedure as a control group. After two months, blood was taken from the pampiniform plexus of the testis which had been treated with artificial varicocele and the testis of control group, then a serologic examination was conducted by a clinical pathologist.

Operation was conducted in general anesthesia by administration of ketamine (100 mg/kg body weight). Both antibiotic (ceftriaxone 50 mg/kg body weight) and analgesia (tramadol) were administered until three days after dissection.

Before and after dissection, all of the rabbits were fed with the same quantity and type of food and in the same time. The food included vegetables (300 g/administration) in the morning and pellet (200 g) in afternoon.

Environment factor was controlled by cleaning the cage every day, administrating prophylaxis antibiotic, and keeping stable room temperature at room temperature between 26-29°C. Room temperature was measured by digital thermometer and monitored each morning. The rabbit cage was exposed to sunlight for 2-3 hours and cleaned each morning.

The technique of dissection applied in making left varicocele was the technique described by Turner TT in 2001. The rabbits were anesthetized by injecting 10 mg/kg body weight IV ketamine through vein in rabbit ears. After the rabbits have already been anesthetized, shaving of hairs in left back, 3-5 cm toward distal costae, was conducted, to form a dissection field of approximately 4-5 cm2 wide. Aseptic and antiseptic treatments were conducted by using betadine 10%, and then the dissection field was closed by sterile drapes. Incision was conducted by using a scalpel no. 13, extending from the direction of head toward tail, parallel to vertebrae, penetrating skin, and then muscle is split by using hemostasis clamp, until retroperitoneal cavity was opened. Left kidney was identified and separated from surrounding tissues carefully, taken out, left renal vein identified, and then its width measured by a caliper, 1 cm medial from renal hilus. Renal vein was bound partly, until it was half of the original renal vein, by using a 3.0 silk. Left kidney was put into its original place, muscle was stitched with a chronic 4.0 interrupted suture, and skin was stitched by a 3.0 continuous suture. For sham operation, the procedure was conducted just until skin incision, and skin was stitched with 3.0 silk to close the wound.

Statistical analysis was performed by using t-Test, to determine the mean difference between research groups. Data processing was conducted by using SPSS for Windows version 16.0.

**RESULTS**

In this research, the weights of both research groups were relatively the same (p > 0.05). The average weight of rabbit was 1475 gr.

In the end of the research, a measurement of the diameter of left internal spermatic vein was conducted. The average diameters of left internal spermatic veins in the sham group and varicocele group were 2.1 ± 0.1 mm and 2.7 ± 0.3 mm.
respectively, which in statistic test the difference were statistically significant (p = 0.019).

**Table 1. Diameters of internal spermatic veins.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Diameter of Internal Spermatic Vein</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average ± SD</td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>2.1 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>Varicocele</td>
<td>2.7 ± 0.3</td>
<td>0.019</td>
</tr>
</tbody>
</table>

From the measurement of the level of testosterone and estrogen hormones in internal spermatic vein or pampiniform plexus of rabbits with varicocele, both testosterone and estrogen hormone showed higher level as compared to the rabbits in control (sham) group. The average level of testosterone hormones in control and varicocele groups were 9.71 ± 2.6 ng/ml and 36.88 ± 6.94 ng/ml respectively. The average level of estrogen hormones in the control and varicocele groups were 0.037 ± 0.005 ng/ml and 0.05 ng/ml. Statistical testing showed a significant difference between the level of testosterone hormone of control group and varicocele group (p < 0.05). Based on a statistic calculation, the levels of estrogen hormones in both research groups were also found significantly different (p < 0.05).

From the results of the calculation of estrogen-testosterone ratio it was found that the estrogen-testosterone ratio of varicocele group was smaller than that of control group by a 1:3 ratio. The estrogen-testosterone ratios of control group and varicocele groups were 0.0039 and 0.0013 respectively. Statistic calculation found that the difference of estrogen-testosterone ratios between research groups were statistically significant (p < 0.05).

**Table 2. Level and ratio of estrogen-testosterone of research groups.**

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Research Groups</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Varicocele</td>
</tr>
<tr>
<td>Estrogen</td>
<td>0.0375</td>
<td>0.0500</td>
</tr>
<tr>
<td>Testosterone</td>
<td>9.7125</td>
<td>36.880</td>
</tr>
<tr>
<td>Ratio</td>
<td>0.0039</td>
<td>0.0013</td>
</tr>
</tbody>
</table>

**DISCUSSION**

The results of research showed that in the pampiniform plexus of testis with varicocele was occurred a hormonal imbalance as compared to the control group. What is the mechanism and what factors that have influence on hormonal imbalance were not determined yet by this research. Some researches have found that in testis with varicocele there occurred static blood flow and Leydig cell dysfunction resulting from hypoxia. Chakraborty J in his research found that in testis with varicocele there occurred static blood flow in testis microcirculation and widening of pore between endotel cells of vessel walls. Ando et al in their research concluded that varicocele causes an increase in temperature in testis, which in turn causes disorder in the function of 17-hydroxyprogesterone aldolase that serves to change 17-hydroxyprogesterone into testosterone. Some studies showed that in varicocele there occurred a decrease in oxygen content and hypoxia in testis tissues that caused damage of ultrastructure, Leydig cell function disorder and decrease in testosterone production. In varicocele, hormonal imbalance may be resulted from either productive or distributional disorder, and this should be further investigated to determined its true pathophysiology.

This research found that the level of testosterone hormone in testis pampiniform plexus of varicocele group was higher than that of control group. According to literature, varicocele theoretically causes a decrease in the production of testosterone hormones so that they are insufficient to keep a normal spermatogenesis process. This theory was support by Sirvent et al, that found in their research a decrease in the number of Leydig cells in the biopsy of testis tissues with varicocele. The results of this research are different with hormonal theory and prior researches. This is probably because the level of testosterone hormones measured in this research had been taken from pampiniform plexus outside the testis, whereas prior researches measured testosterone hormones inside testis tissues. From literature it is known that the level of intratesticular testosterone hormone is 100 times higher than the level in peripheral circulation. In varicocele, in addition to factors of a decrease in production due to dysfunction and a decrease in number of Leydig cells, maybe there are some other factors such as static blood flow that influences the level of testosterone hormone in pampiniform plexus.

**CONCLUSION**

Estrogen-testosterone ratio in the pampiniform plexus of rabbits with varicocele was smaller than that of normal rabbits.
REFERENCES