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Evaluation of Serum Galectin-3 level in Varicocele Patients

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Abstract
Background: Varicocele is connected with oxidative damage. Carbohydrate binding lectin galectin-3 has a role in regulating oxidative stress, inflammation, fibrosis, and angiogenesis. The purpose of this study is to examine galectin-3 levels between patients with a varicocele and healthy controls. Methods and Subjects: Group (A) consisted of 30 patients with oligoasthenoteratozoospermia, Group (B) consisted of 30 patients with normozoospermia, and Group (C) consisted of 20 healthy persons of the same age range as the patients. All subjects had their blood galectin-3 quantified using ELISA, and their varicocele severity was determined using the standard Dubin grading system. The age, smoking status, and body mass index of the patients and controls did not vary significantly. With respect to galectin-3, there was a statistically significant difference between the patient and control groups. There was a statistically significant (P 0.001) difference in serum galectin levels between the groups. An additional post hoc analysis showed that it was considerably greater in group A compared to groups B and C. Further, group B had a much greater rate than group C. The results of the present investigation revealed that serum galectin-3 levels were higher in individuals with varicoceles and were associated with semen parameters.

Key words: Varicocele, Serum Galectin-3 level, Healthy controls

1. Introduction

A varicocele develops when the internal spermatic vein and pampiniform venous plexus in the spermatic cord enlarge abnormally, causing a bulge in the scrotum [1].

Increased reactive oxygen species (ROS) generation in the semen and impaired antioxidant capacity have both been linked to varicocele[2].

Humans have a gene called LGALS3 on chromosome 14 (locus q21-q22) that codes for the protein galectin 3 (Gal-3), which is 31 kDa and detects the N-acetyl-lactosamine structure of various glycoconjugates [3].

3 Galactose 3-phosphate (Gal-3) plays a crucial role in a wide variety of biological processes, such as cell proliferation, apoptosis, pre-mRNA splicing, differentiation, transformation, angiogenesis, inflammation, fibrosis, and host defence [4].

Gal-3 is a gene that seems to be hormone dependent, since it is only expressed in the mature Sertoli cells and Leydig cells of the human testis [5].

We set out to examine galectin-3 levels between healthy people and those with varicoceles in this research.

2. Subjects And Methods

Sixty male patients with a history of varicocele complaints were compared to twenty healthy men and women of similar age and sex who volunteered to take part in the research. In order to be disqualified, participants needed to meet these conditions: Patients with secondary varicoceles, patients younger than 18, and patients with chronic conditions such diabetes, hypertension, liver cirrhosis, and renal failure are not good candidates for surgery. Group (A) consisted of thirty patients diagnosed with oligoasthenoteratozospermia, group (B) consisted of thirty patients diagnosed with normozoospermia, and group (C) consisted of twenty age-matched healthy persons serving as a control group.

After a thorough physical examination, we were able to ascertain the varicocele's severity using the Dubin grading system [6].

Patients and healthy controls alike had 5 ml of venous blood drawn while fasting so that serum galectin-3 levels could be measured using an ELISA Kit.

3. Statistical Analysis

Data management and statistical analysis were done using SPSS vs.25. (IBM, Armonk, New York, United States). Numerical data were summarized as means and standard deviations or medians and ranges. Categorical data were summarized as numbers and percentages. Numerical data were assessed for normality using the Kolmogorov–Smirnov test, Shapiro–Wilk test, and direct visualization of the data. Comparisons between the three groups were done using one-way ANOVA test or Kruskal Wallis test for normally and non-normally distributed numerical data. Categorical data were compared using the Chi-square test. Correlation analysis was done between serum galectin and other parameters using Pearson’s or Spearman’s correlation. Serum galectin-3 was compared regarding background variables as smoking, obesity, and associated diseases using the Mann-Whitney U test. Multinomial logistic regression analysis was done for using galectin-3 for the prediction of varicocele. The odds ratio and 95% confidence interval were

calculated. All P values were two-sided. P values less than 0.05 were considered significant.

4. Results

There were no significant differences between the three study groups regarding age (***P***=0.058), BMI (***P***=0.727), smoking (***P***=0.179) and obesity (***P***=0.701) **(Table 1)**

Table (1) General characteristics in study groups

		Group I (n = 30)	Group II (n = 30)	Group III (n = 20)	P value
Age (Years)	Mean ±SD	35 ±11	29 ±8	31 ±9	0.058
BMI	Mean ±SD	26.3 ±5.8	25.4 ±5.7	25 ±5	0.727
Smoking	n (%)	11 (36.7)	5 (16.7)	7 (35.0)	0.179
Obesity	n (%)	8 (26.7)	6 (20.0)	6 (30.0)	0.701

One-way ANOVA was used for age & BMI. Chi-square test was used for categorical data Disease duration and varicocele grade showed no significant differences between both groups; P values were 0.66 and 0.268, respectively. The most frequent associated diseases in group I were Azoospermia, Hydrocele, and Inguinal Hernia (10% for each). In group II, the most frequent associated disease was hydrocele (13.3%) **(Table 2)**.

Table (2) Disease characteristics in varicocele groups

			Group I (n = 30)	Group II (n = 30)	*P*
Disease duration (month)	Median (range)		3 (1 - 12)	3 (1 - 8)	0.66
Varicocele grade	Grade I	n (%)	9 (30 %)	4 (13.3 %)	0.268
	Grade II		11 (36.7 %)	12 (40 %)	
	Grade III		10 (33.3 %)	14 (46.7 %)	

Mann-Whitney U test was used for disease duration. Chi-square test was used for varicocele grade NA = not applicable

The median total sperm count showed an overall significant difference between the three groups (***P*** <0.001). Post hoc analysis revealed that it was higher significantly in group III (169 million/ejaculate) than groups I (25.5 million/ejaculate) and II (48 million/ejaculate). Also, it was higher significantly in group II than group I.

The median sperm concentration showed an overall significant difference between the three groups (***P*** <0.001). Post hoc analysis revealed that it was higher significantly in group III (69.5 million/ml) than groups I (10 million/ml) and II (23.5 million/ml). Also, it was higher significantly in group II than group I.

The median percent of progressive motility showed an overall significant difference between the three groups (***P*** <0.001). Post hoc analysis revealed that it was higher significantly in group III (50%) than groups I (10%) and II (37%). Also, it was higher significantly in group II than group I.

The median percent of non-progressive motility showed an overall significant difference between the three groups (***P*** <0.001). Post hoc analysis revealed that it was lower significantly in group I (10%) than groups II (20%) and III (25%), with no significant difference between groups II & III.

The median percent of immotile sperms showed an overall significant difference between the three groups (***P*** <0.001). Post hoc analysis revealed that it was higher significantly in group I (72%) than groups II (45%) and III (23%). Also, it was higher significantly in group II than group III.

The median sperm abnormal forms showed an overall significant difference between the three groups (***P***<0.001). Post hoc analysis revealed that it was higher significantly in group I (98%) than groups II (65%) and III (12%). Also, it was higher significantly in group II than group III. All these data were shown in **Table (3)** and **Chart (1)**.

Table (3) Semen analysis in patients and control groups

		Group I (n = 30)	Group II (n = 30)	Group III (n = 20)	*P*
Semen volume (ml)	Median (range)	2 (0.5 - 20)	2.5 (0.3 - 6)	2.8 (2 - 4.2)	0.106
Liquefaction (minutes)	Median (range)	30 (15 - 70)	20 (15 - 70)	18 (15 - 30)	0.156
Total sperm count (M/ejaculate)	Median (range)	25.5 (0 - 40)[a]	48 (42 - 90)[b]	169 (98 - 440)[c]	<0.001
Sperm concentration (M/ml)	Median (range)	10 (0 - 14)[a]	23.5 (17 - 90)[b]	69.5 (34 - 110)[c]	<0.001
Progressive motility (%)	Median (range)	10 (0 - 27)[a]	37 (10 - 60)[b]	50 (40 - 84)[c]	<0.001
Non-progressive (%)	Median (range)	10 (0 - 30)[a]	20 (6 - 30)[b]	25 (3 - 30)[b]	<0.001
Immotile (%)	Median (range)	72 (62 - 100)[a]	45(30 - 75)[b]	23 (10 - 48)[c]	<0.001
Pus cells (>10 cells/hpf)	n (%)	14 (46.7)	10 (33.3)	7 (35.0)	0.527
Abnormal forms (%)	Median (range)	98 (0 – 100)[a]	65 (30 - 91)[b]	12 (10 – 33)[c]	<0.001

Kruskal Wallis test was used for numerical data. Chi-square test was used for pus cells. Post hoc was done using the Bonferroni method, and different letters indicate significant pair.

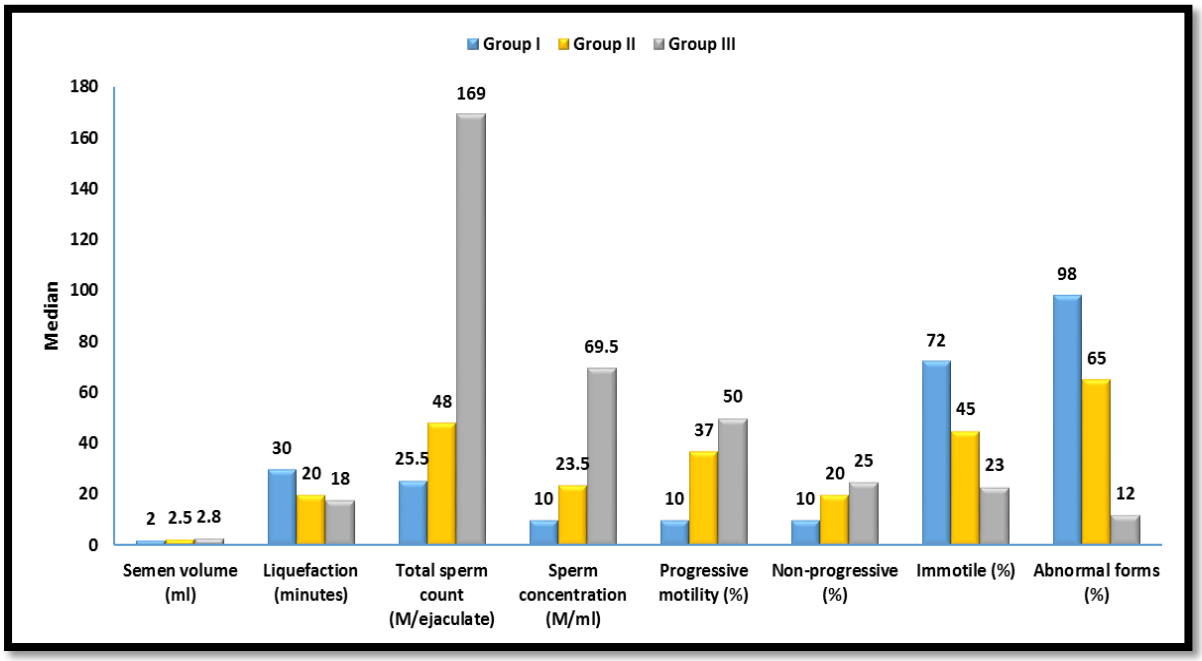

Chart (1) Semen analysis in patients and control groups

5. Discussion

About 40% of men with primary infertility and 80% of men with secondary infertility have varicoceles, but only 12% of men with normozoospermia have them [7]. Varicocele has a varying influence on male fertility, although the underlying process is not well understood. Growth of the testes and the quality of the sperm might be impacted by varicocele [8].

Damage to spermatogenesis is caused by the testicular heat, hypoxia, and oxidative stress that are brought on by a varicocele [9].

There is evidence that inflammation hinders sperm synthesis by increasing levels of inflammatory cytokines and reactive oxygen species (ROS). Semen from infertile males has been shown to contain altered expression of cytokines such IL-6, IL-8, and IL-10 [10].

As a -galactoside-binding lectin, galectin-3 has a role in cell proliferation, apoptosis control, inflammation, fibrosis, and host defence, among many other biological functions and organ systems [11].

Superoxide dismutase, an antioxidant enzyme, was able to counteract the oxidative stress caused by galectin-3 by inhibiting the generation of the superoxide radical (O2) from cultured mast cells [12].

ObjectivesSerum levels of galectin-3 were measured in patients with varicoceles and compared to those in healthy controls. Stronger associations were found between galectin-3 and the semen parameter.

On measures of age, body mass index, and smoking status, there were no statistically significant differences across the research groups. The correlation between varicocele and body mass index has shown contradictory findings. There was a negative correlation between body mass index and the incidence of varicocele, according to several studies. According to May et al. [13], men with greater body mass indexes are less likely to experience the nutcracker phenomenon because their extra fat acts as a cushion, reducing the compression of the left renal vein [14]. While other research has revealed no correlation between the two [15].

Thirty individuals with varicoceles but normal semen parameters comprised group (II) in the current investigation. Studies by Fariss et al. [16] comparing semen analyses in men with and without a varicocele support this. Studies comparing males with and without varicoceles have revealed no statistically or clinically significant differences in mean sperm count, motility, or morphology. Similar research has studied semen parameters in infertile men with and without a varicocele [17, 18]. Expectedly, semen parameters were frequently aberrant in both groups of men; if the semen analysis had been normal, the men would not have been referred for further investigation. However, there is no clear evidence from these studies that infertile men who have a varicocele have worse semen quality than infertile men who do not (idiopathic infertility).

Serum levels of galectin-3 were measured in patients with varicoceles and compared to levels in healthy controls for the first time in this research. The median serum galectin-3 levels of all three groups were significantly different from one another, notwithstanding the variations in semen quality. In addition, individuals with varicoceles and aberrant semen parameters had greater levels of serum galectin-3 than those with normal semen.

The potential of galectin-3-binding protein in semen as a non-invasive biomarker in infertile men with aberrant semen parameters has been investigated in two earlier investigations. Oligoasthenoteratozoospermia was shown to have lower levels of galectin-3-binding protein [19, 20]

6. Conclusion

Galectin-3 is a putative regulator of inflammation in the male reproductive system and might be viewed as a local marker of inflammation, and the presence of greater levels of galectin-3 in plasma of varicocele patients supports its relationship with free radicals and inflammation.

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