Measurement of Leptin Hormone in Serum and Seminal Plasma Before & After Varicocelectomy

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Abstract:Introduction: Varicocele is the most common cause of male infertility. Varicocelectomy significantly improves sperm concentration and motility in infertile men with palpable varicocele and abnormal preoperative semen parameters. In adults, leptin stimulates gonadotrophin-releasing hormone (GnRH) secretion and exerts indirect effects on the gonads via neuropeptide Y. Leptin might have local effects on spermatogenesis.
Aim of the study: To measure leptin hormone in serum and seminal plasma before and after varicocelectomy and find its effect on semen parameters.
Patients and Methods: This comparative study was conducted on 22 infertile men presented with clinical varicocele.All patients were subjected to: Complete history taking, General examination, Local examination , scrotal duplex ultrasound and Laboratory investigations includingsemen analysis and detection of leptin in serum and seminal plasma.
Results: This study showed no statistical significant difference between serum leptin before and after varicocelectomy and statistically significant difference between seminal leptin before and after varicocelectomy. Regarding sperm concentration and motility, there was no statistical significant difference between sperm concentration and non-progressive motility before and after varicocelectomy, but there was statistically significant difference between progressive motility before and after varicocelectomy.
Conclusion: The seminal palasma leptin significantly decreased after varicocelecetomy , but serum leptin shows no statistically significant difference after varicocelectomy.There was statistically significant negative correlation between serum leptin and progressive motility before and after varicocelectomy.
[A.R. Elshahid, A.S. Al-Adl, N.F. Esmailand M.I. Elsayed **Measurement of Leptin Hormone in Serum and Seminal Plasma Before & After Varicocelectomy.** *Nat Sci*2018;16(8):45-52].ISSN 1545-0740 (print); ISSN 2375-7167 (online).http://www.sciencepub.net/nature.6. doi:10.7537/marsnsj160818.06.

Keywords: Measurement; Leptin; Hormone; Serum; Seminal; Plasma; Varicocelectomy

1. Introduction:

Varicocele (also known as varicose seal), is an abnormal enlargement of the pampiniform venous plexus in the scrotum. This plexus of veins drains the testicles. The testicular blood vessels originate in the abdomen and course down through the inguinal canal as part of the spermatic cord on their way to the testis. Upward flow of blood in the veins is ensured by small one-way valves that prevent backflow. Defective valves, or compression of the vein by a nearby structure, can cause dilatation of the testicular veins near the testis, leading to the formation of a varicocele **(1).**

Seminal plasma leptin is negatively correlated with spermatogenesis and sperm motility in varicocele patients **(3).**Leptin may be related to spermatogenesis dysfunction caused by varicocele and other etiologies**.** The concentration of leptin in seminal plasma, inversely related to sperm concentration and motility. It would be significantly elevated in the infertile patients with oligozoospermia, asthenozoospermia and varicocele **(8).**

The aim of this work was to measure leptin hormone in serum and seminal plasma before and after varicocelectomy.

2. Patients and Method:

This study was conducted on twenty two men presented with clinical varicocele with infertility and fulfilling the inclusion criteria in the absence of any of the exclusion criteria. They were recruited from the attendance of Andrology Unit atAlhussien University Hospital.

Inclusion criteria:

All included subjects are with the following criteria:

1. Infertile men of one-year duration.
2. Patients with bilateral or unilateral varicocele ≥ grade II.
3. Patients presented with abnormal semen parameters.
4. Patients with normal testicular size.

Exclusion criteria:

Exclusion criteria included the following:

1. Pyospermia and BMI > 30.
2. Factors that may affect the metabolism of serum leptin in blood (Insulin thrapy, corticostroides and hormonal therapy).

All patients were subjected to:

I) Complete history taking including:

1. Personal history.
2. Present history:

a. Sexual history

including criteria of androgen deficiency such as loss of libido, depression and erectile dysfunction.

b. Medical history

including chronic diseases, drugs affecting metabolism of leptin.

c. Infertility history

including infertility more than one year with regular sexual intercourse without use of any contraceptives.

3. Past-history

including history of undescended testis, mumps orchitis, testosterone replacement therapy or antiestrogen or aromatase enzyme inhibitor, uncontrolled diabetes, hypertension, chemotherapy or radiotherapy and history of hepatic diseases.

II) Examination:

1. General examination including mean arterial blood pressure, weight, height, BMI, waist circumference, hair distribution.
2. Local examination:

- Inspection:

Pubic area for hair distribution, defect in hair growth and scars of operations.

- Palpation:

Penile examination for presence of Peyronie's disease, penile deviations and micropenis.

- Testicular size

by examination and duplex ultrasound. Consistency, vas palpation, presence and degree of varicocele and any other abnormalities.

- Varicocele was detected by physical examination and confirmed by color flow Doppler ultrasound examination.

II) Laboratory investigations:

- Semen analysis was done before and 6 months after varicocelctomy .
- Serum and seminal plasma Leptin was assessed by ELISA before and 6 months after varicocelectomy BY (Human Leptin ELISA Kits, China).

- Color flow Doppler ultrasound testicular examination for detection of varicocele and testicular size.
- Semen analysis 6 months after varicocelectomy was done.

IV) All participants were under went subinguinal bilateral varicocelectomy .

Statistical analysis:

Data were analyzed using Statistical Program for Social Science (SPSS) version 15.0. Quantitative data were expressed as mean± standard deviation (SD). Qualitative data were expressed as frequency and percentage.

The following tests were done:

- **T test (T):** was used to compare between two means.
- **Pearson's correlation coefficient (r):** test was used for correlating data.
- **Probability (P-value):**
- P-value < 0.05 was considered significant.
- P-value < 0.001 was considered as highly significant.
- P-value > 0.05 was considered insignificant.

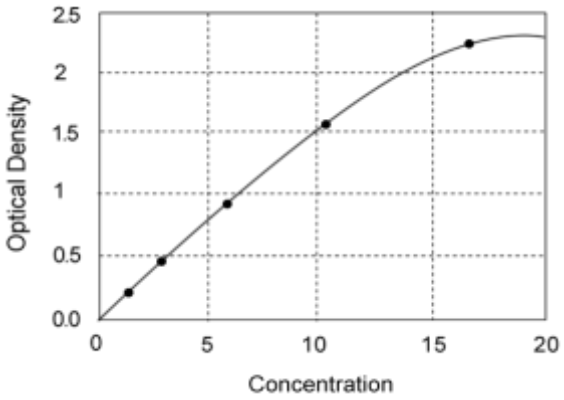

Figure (1): Calculation of results

3. Results:

In this study, patients mean age was 31.9 ± 7.7 years, 73% of patients were grade II varicocele while 27% of them were grade III.The seminal palma leptin showed a statistically significant decrease after varicocelectomy(p-value < 0.05), also serum leptin was statistically insignificant decrease after varicocelectomy.there was no statistical significant difference (p-value > 0.05) between sperm concentration and non-progressive motility before and after varicocelectomy, but there was statistically significant difference (p-value < 0.05) between progressive motility before and after varicocelectomy.

There was a statistically significant (p-value < 0.05) negative correlation between serum leptin and progressive motility before varicocelectomy and non-statistically significant (p-value > 0.05) correlations betweenseminal leptin and other studied parameters before and after varicocelectomy.(tables 1-7), (figure 2-6).

Table (1):Description of age, grade of varicocele and duration after operation in studied patients.

Groups / Variables		Studied patients (N = 22)
Age (years)	**Mean**	31.9
	±SD	7.7
Grade of varicocele	**II**	16 (73%)
	III	6 (27%)
Duration after operation	**Mean**	6.2
	±SD	0.4

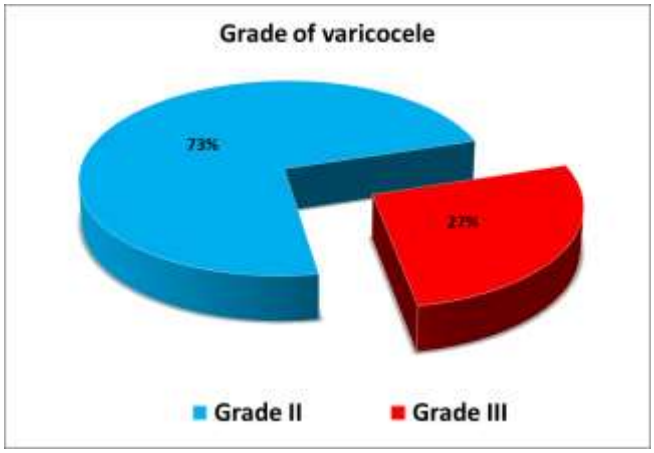

Figure (2):Description of grade of varicocele in studied patients.

Table (2):Comparison between serum, seminal leptin before and after varicocelectomy.

Groups / Variables		Before operation (N = 22)	After operation (N = 22)	p-value
Serum leptin	**Mean**	172	92.5	0.1
	±SD	236.01	16.3	
Seminal leptin	**Mean**	124.4	91.2	**0.004***
	±SD	45.9	18.2	

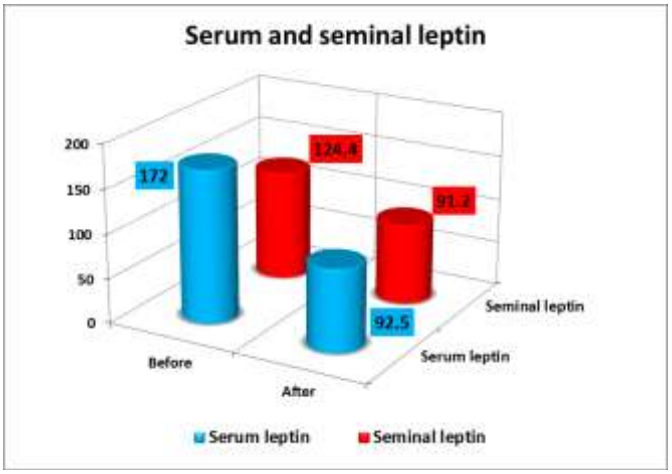

Figure (3):Comparison between serum, seminal leptin before and after operation.

Table (3): Comparison between sperm concentration, progressive motility, non-progressive motility before and after varicocelectomy.

Groups / Variables		Before operation (N = 22)	After operation (N = 22)	p-value
Sperm Conc.	**Mean**	8.07	15.2	0.5
	±SD	8.02	14.9	
Progressive motility	**Mean**	14.8	27	**0.03***
	±SD	12.9	22.3	
Non-Progressive motility	**Mean**	18.7	21.6	0.7
	±SD	28.1	27.2	

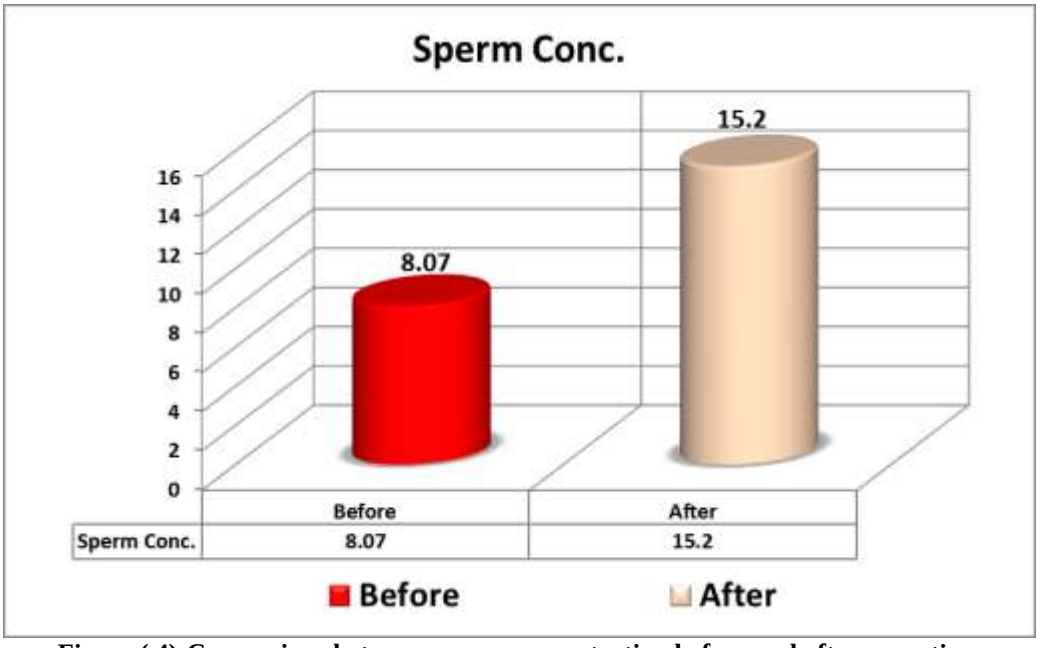

Figure (4):Comparison between sperm concentration before and after operation.

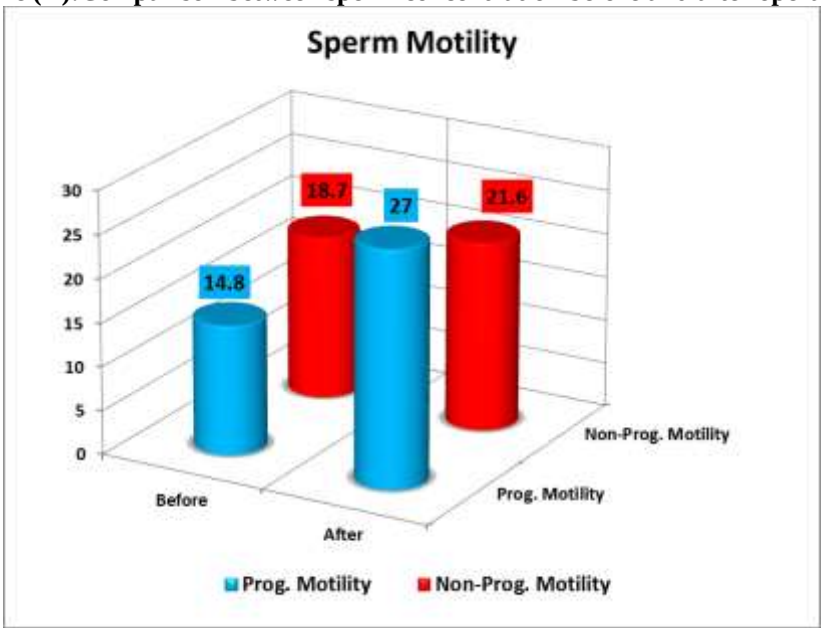

Figure (5):Comparison between sperm motility before and after operation.

Table (4): Correlation study between serum leptin and other studied parameters in studied patients before operation.

Before operation / Parameters	Serum Leptin	
	(r)	p-value
Serum leptinvs seminal leptin	- 0.3	0.07
Serum leptinvs sperm Conc.	0.4	0.02
Serum leptinvsProg. motility	- 0.09	**0.03***
Serum leptinvs Non-Prog. motility	- 0.1	0.5
Serum leptinvs Abnormal forms	- 0.1	0.5

Table (5): Correlation study between seminal leptin and other studied parameters in studied patients before operation.

Before operation / Parameters	Seminal Leptin	
	(r)	p-value
Seminal leptinvs Serum leptin	- 0.3	0.07
Seminal leptinvs sperm Conc.	0.1	0.4
Seminal leptinvsProg. motility	0.2	0.4
Seminal leptinvs Non-Prog. motility	- 0.1	0.6
Seminal leptinvs abnormal forms	- 0.4	0.05

Table (6): Correlation between serum leptin and other studied parameters in studied patients after operation.

after operation / Parameters	Serum Leptin	
	(r)	p-value
Serum leptinvs seminal leptin	0.3	0.1
Serum leptinvs sperm Conc.	- 0.2	0.4
Serum leptinvsProg. motility	- 0.3	0.2
Serum leptinvs Non-Prog. motility	0.02	0.9
Serum leptinvs abnormal forms	0.7	**< 0.001***

Table (7): Correlation between seminal leptin and other studied parameters in studied patients after operation.

after operation / Parameters	Seminal Leptin	
	(r)	p-value
Seminal leptinvs Serum leptin	0.3	0.1
Seminal leptinvs sperm Conc.	- 0.03	0.8
Seminal leptinvsProg. motility	0.3	0.2
Seminal leptinvs Non-Prog. motility	- 0.04	0.8
Seminal leptinvs abnormal forms	- 0.2	0.2

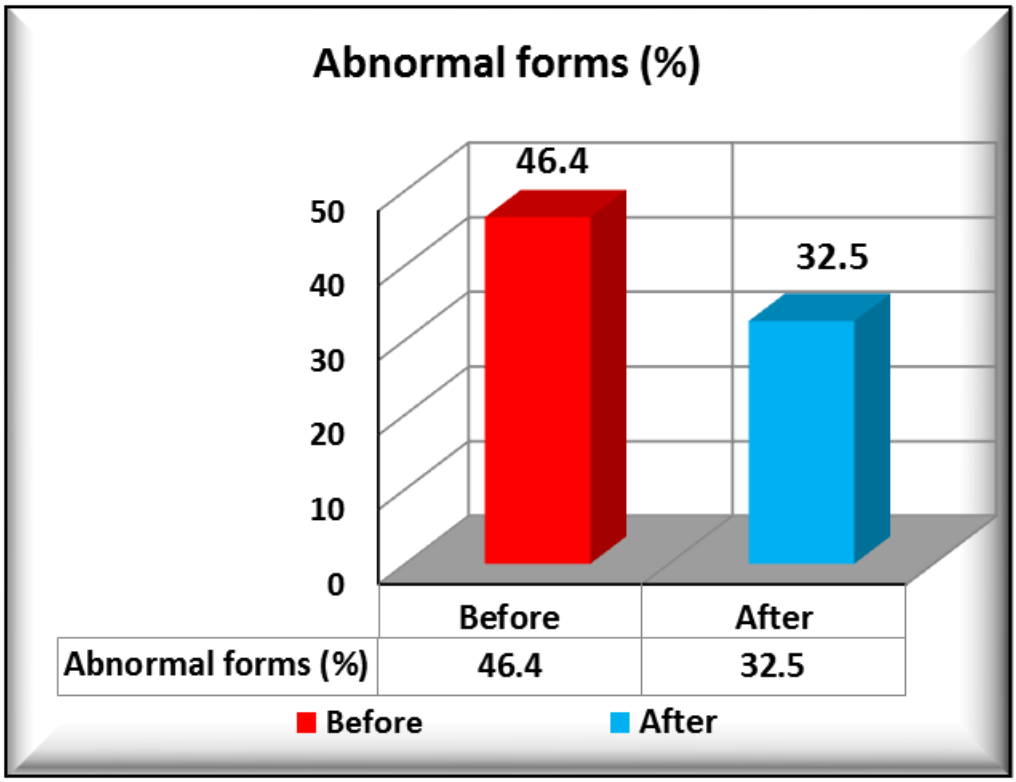

Figure (6):Comparison between abnormal forms before and after varicocelectomy

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4. Discussion:

The association of varicocele and male infertility has been known for more than a century and varicocele is now regarded as the leading cause of male infertility **(10).**

varicocele-related spermatogenic dysfunction was closely related to increased mRNA and protein expression of leptin in the experimental varicocele rat testis**(3).**

Although the participation of leptin in female reproduction is well established, its role in male reproduction is under investigation **(2)**.

So, the aim of this work was to measure leptin in serum and seminal plasma before and after varicocelectomy.

In this study, patients mean age was 31.9 ± 7.7 years, 73% of patients were grade II varicocele while 27% of them were grade III.

Wang et al,investigated the effect of leptin on spermatozoa apoptosis. Spermatozoa from 40 normospermicmen were used as controls. Routinesemen analysis and seminal plasma leptin levels were measured.The spermatozoa from a total of 184 patients were analysed.Of these, 74 patients were diagnosed with varicocele, withan average age of 29.76 ± 13.18 years. Seventy patientswere diagnosed with leucocytospermia, and the averageage of this group was 28.18 ± 11.64 years **(12)**.

This study showed no statistical significant difference (p-value > 0.05) between serum leptin before and after varicocelectomy and statistically significant difference (p-value < 0.05) between seminal leptin before and after varicocelectomy. Our results in agreement with ***Ni***et al which investigated the expression and the exact role of leptin under hypoxic conditions in human testis. After varicocelectomy, in the post-6m-operation group seminal plasma leptin (2.35±0.78ng/L, P<0.05) was significantly lower when compared with the pre-operation group.

Serum leptin demonstrated no differences (P>0.05) among groups**(8)**.

Regarding sperm concentration and motility, there was no statistical significant difference (p-value > 0.05) between sperm concentration and non-progressive motility before and after varicocelectomy, but there was statistically significant difference (p-value < 0.05) between progressive motility before and after varicocelectomy.

Koda et al,demonstrated that leptin can be detected in human seminal plasma which may comefrom blood and testis itself. The concentration of leptin in seminal plasma, inversely related to spermconcentration and motility, would be significantly elevated in the infertilepatients with

oligozoospermia, asthenozoospermia and varicocele**(6)**.

Wang et al,compared pH, sperm density and sperm motility between the varicocele, leucocytospermia and control groups. There were no obvious differences in pH among the three groups. When sperm density was analysed, the varicocele group was significantly lower than the control group. When sperm motility was analysed, the varicocele and leucocytospermia groups were both significantly lower than the control group. The seminal plasma leptin levels in the varicocele and leucocytospermiagroups were higher than those measured inthe control group, and these differences were statisticallysignificant**(12)**.

Depending on these findings, we detected statistically significant (p-value < 0.05) negative correlation between serum leptin and progressive motility before varicocelectomy and non-statistically significant (p-value > 0.05) correlations betweenseminal leptin and other studied parameters before and after varicocelectomy highly statistically significant correlation between serum leptin and abnormal forms after the operation .

These findings are incopatable with ***Camina et al,***whoreported that no correlation between leptin concentrations in semen and the physical characteristics of semen samples or physical characteristics of sperms, such as concentration, motility, vitality, or morphology**(2)**.

The relationship between serum leptin and the sperm count may be explained by the dysfunction of testicular epithelium which may occur in azoospermic and oligozoospermic patients with decreased testicular production of testosterone which is inversely correlated with serum leptin level.

Francois and chen,have found that seminal plasma leptin is negatively correlated with spermatogenesis and sperm motility in varicocele patients**(5).**

Ni et al,found negative correlations between seminal plasma leptin and sperm concentration, as well as progressive motility**(8)**.

In a study done by ***Wang et al,*** sperm apoptosisrate and sperm density, and sperm apoptosis rate andsperm motility had significant negative correlations, and a significantpositive correlation with seminal plasma leptin levelsin the varicocele group**(12)**.

Conclusion and recommendations:

Experimental studies to clarify the mechanism of action of leptin on male gonads should be done.

Serum leptin should be studied in different conditions associated with azoospermia or oligozoospermia.the seminal palasma leptin significantly decreased after varicocelecetomy , also serum leptin insegnfecantly decreased after varicocelectomy.There wasstatistically significant negative correlation between serum leptin and progressive motility before and after varicocelectomy.

The results of this study are suggestive of a link between leptin and male reproduction. This link requires further detailed studies to be clear.

Varicocele could increase the concentration of leptinin seminal plasma, inversely related to sperm concentration and motility, whichcould be effectively diminished via varicocelectomy.

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6/20/2018