Role of son elastography in predicting positive sperm retrieving in patients with azoospermia

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ABSTRACT

BACKGROUND:
Infertility is an important community problem & about 10-30 % is due to azoospermia.
Many causes of azoospermia were found.
It's either obstructive or non-obstructive.
When testicular biopsy is negative, this means that it's a non-obstructive azoospermia.
When testicular biopsy is positive, this means that it's either an obstructive or a non-obstructive azoospermia.
Elastography is a new ultrasound modality which simply could evaluate the internal elasticity of the testis & finally give an idea about the presence or absence of sperms.
AIM OF THE STUDY:
To evaluate the validity of sonoelastography in predicting positive sperm retrieving in patient with azoospermia.
PATIENTS AND METHODS:
This study is a cross sectional study which performed in Al-Sader Teaching Hospital / fertility center & radiology department during the period from 1st September 2017 to 1st September 2018.
We had studied (98 patients /196 testes) with azoospermia by B-mode U/S, Doppler study (RI) & Elastography (strain ratio) , then the patients return back to the fertility center to do a biopsy.
RESULTS:
198 testes were examined by radiologist, from it only (136) testes were subjected to biopsy.
Testicular volume & strain ratio were the most important factors which suggest that the testis will give a positive or negative sperm retrieving.
Low volume + high strain ➔ NSR (bad prognosis).
High volume + low strain ➔ PSR (good prognosis).
These findings of SR gives a
Sensitivity of 75.7%,
Specificity= 93%,
Positive predictive value= 78%
Negative predictive value= 92 and
Accuracy of 89%.
Conclusion:
Real-time elastography is a promising imaging method with greatPotential for the differential diagnosis of azoospermi

Keywords:

Acknowledgments

No one can start & complete anything withoutthe help of our generous God, great thanks God for kind help forever.

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1.1. Introduction:

The World Health Organization (WHO) and the American Society for

Reproduction Medicine Practice Committee (ASRM) defines infertility as that "In ability of sexually active, non- contraceptive couples to achieve spontaneous pregnancy after one year of non- protective sexual intercourse "(1).

20 % of male infertility situations may suffer from azoospermia,

Whicheither obstructive or non-obstructive(1).

Definition**:**

1.1.1. Azoospermia:

It means that ' absence of sperm in ejaculate of at least two seminal analysis,andof two semen samples obtained more than two weeks apart (2, 3).

It is either obstructive or non-obstructive.

Many causes of azoospermia which are further classified to pre - , post- & testicular causes as shown in figure 1.

1.1.2. Causes of azoospermia:

Azoospermia may be due to inadequate hormonal Stimulation, impaired spermatogenesis or an obstruction.In the majority of cases, the assessment of both physical and Laboratory information, including semen volume, testicular Volume, the presence of bilateral vas deferens and serum FSH level, will aid in the differentiation between the three Categories (4)

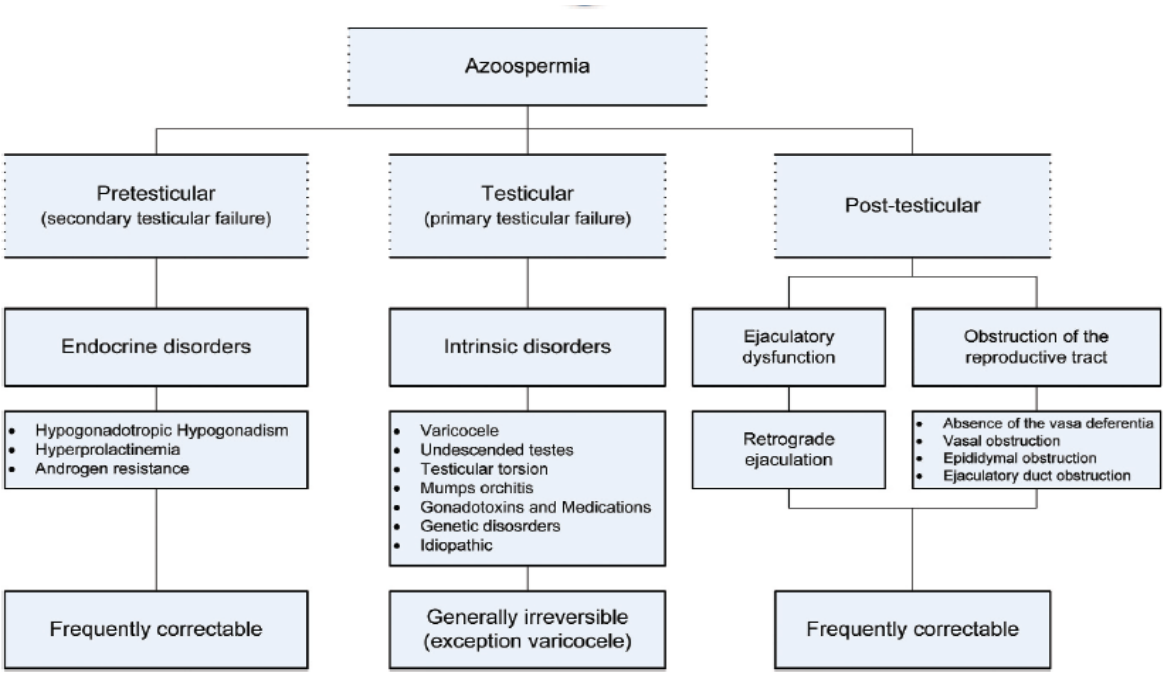


Fig (1) showsdifferent causes of azoospermia (4).

1.1.2.1. Pre-testicular causes (Hormones):

Reproductive axis of hormones known as hypothalamic –pituitary –gonadal axis, consisting of hypothalamus, pituitary gland & testes.

Any disturbance in this sequence can leads to infertility.

E.g. in primary hypogonadism, the gonadotropin (FSH & LH) levels are increased a condition called "hyper-gonadotrophic hypogonadism".

While in secondary hypogonadism, the gonadotropin levels are low a condition called "hypogonadotrophic hypogonadism".

In another wards ,if there is a failure in releasing gonadotropin releasing hormone properly this may lead to lack of testosterone production & cessation sperm synthesis in a condition known as hypogonadotrophic hypogonadism(5).

FSH level measurements is vital to differentiate between hyper - & hypogonadotrophic hypogonadism (6).

Normal FSH level in patients with azoospermia with normal volume testes may indicate obstruction of sperm transport (6).

Estimation of FSH level is not required in men with sperm concentration more than 10 million / ml and normal testicular volume (7).

1.1.2.2. Testicular causes:

A-Varicocele.

Currently, there is convincing evidence in the literature that varicocele produce a progressive harmful effect on the testis, and varicocelectomy has been shown to prevent the progressive decline in testicular function and reverse the damage (8, 9,10). Additionally, varicocele repairs have been documented to improve pregnancy rates and ART outcomes (8,9,10).

B-Undescended testes:

Undescended testes are the most common genital malformation in boys and are noted in 2.7% of newborns and up to 0.8% of 1-year-olds (11). It is important to differentiate cryptorchid testes from retractile testes, a circumstance involving hyperactive cremasteric muscles that cause the testes to periodically reside in the inguinal canal or high scrotum. Suggested mechanisms for cryptorchidism-induced subfertility include testicular digenesis, an impaired endocrine axis, immunologic damage, and obstruction (12).

C-Testicular torsion:

Testicular torsion occurs in approximately 1:4,000 males before the age of 25 years (13). This disorder demands immediate surgical exploration, and the risks of non-operative management are well documented (14). Testicular preservation is usually achieved if surgical exploration is performed within 6 hoursafter the onset of symptoms.

D-Mumps orchitis:

Since the introduction of a vaccine against the mumps virus, there has been a reduced risk of mumps and its complications. On the other hand, mumps orchitis should still be suspected in cases of scrotal swelling, as there has been a recent increase in mumps orchitis among pubertal and post-pubertal males (15).

Pubertal mumps orchitis occurs unilaterally in 67% of patients and bilaterally in 33% (16). Testicular atrophy occurs in 36% of those affected bilaterally, whereas infertility occurs in just 13% (17). However, pre-pubertal mumps orchitis has little effect on future fertility (16).

E-Gonadotoxins and medications:

Drugs and medications may harm male fertility through four distinct mechanisms:

1) Direct gonadotoxic effects, 2) alteration of the hypothalamic-pituitary-gonadal (HPG) axis, 3) ejaculation dysfunction, and 4) reduction in libido. Meanwhile, Gonadotoxins affect spermatogenesis by direct injury to germ cells in the testis or by interfering with the function of the Sertoli cells (18).

1.1.2.3. Post-testicular:

Post-testicular causes of azoospermia are due to either the obstruction of sperm delivery or ejaculatory dysfunction.

The clinical management of obstructive azoospermia depends on its cause and also must take into account any coexisting infertility factors in the female partner (19).

The most important causes are:

A-Absence of the vasa deferentia:

Congenital bilateral absence of the vas deferens (CBAVD) is found in 1% of infertile men and in up to 6% of those with obstructive azoospermia (20).

B-Vasal obstruction:

The most frequent cause of non-purposeful vasal obstruction is inadvertent injury during the performance of a hernia repair. This complication more frequently occurs when performed in infancy but can occur after any inguinal procedure where the vas and cord are manipulated (21).

C-Epididymal obstruction:

Young's syndrome is a triad of disorders that encompasses chronic sinusitis, bronchiectasis and obstructive azoospermia (22).

1.2. Anatomy of testis

1.2.1. Gross anatomy:

Testes are the primary reproductive organs of the male, with sperm & testosterone hormone production functions.

They are oval in shape, with upper & lower convex poles, convex anterior & nearly straight posterior surfaces.

They are suspended in the scrotum by scrotal tissues including the dartos muscle and spermatic cords, lying obliquely in the scrotal sac, with the upper pole tilted antero - laterally and the lower postero-medially(23)

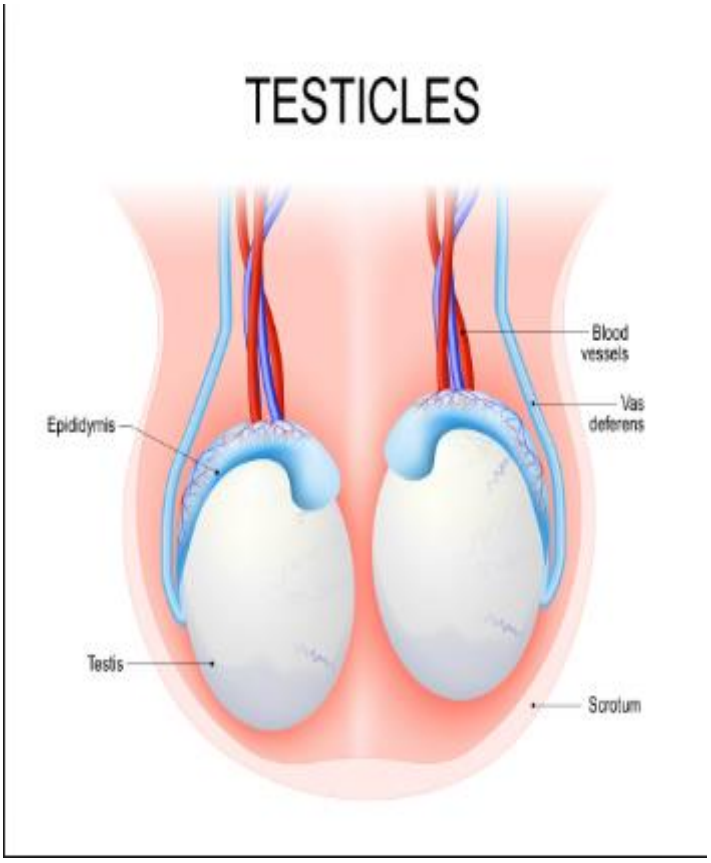


Fig (2). Relation between testes& epididymis(23).

Average volume of healthy young male testis is 20 cc (which decrease in elderly),with dimensions of 4–5 cm in length, 2.5 cm in breadth and 3 cm in antero-posterior diameter(measurement of testicular volume is very important to evaluate fertility ,as the sperm forming cells & the seminiferous tubules (the spermatogenic region of the testis) constitute about 80 % of the testicular volume ,so it is reflect a rough estimate of the

spermatogenic cell capacity) ,consistency of the testis is of great value to determine fertility capacity ,a soft testis is likely to reflect degenerating or shrunken spermatogenic components within the seminiferous tubules(23)

1.2.2. Internal structure:

Each testis covered by tightly adherent capsule, the tunica albuginea which thickened posteriorly to form a fibrous septa "the Mediastinum testis "(which make the entrance of Blood vessels, lymphatic and the genital ducts to the testis).

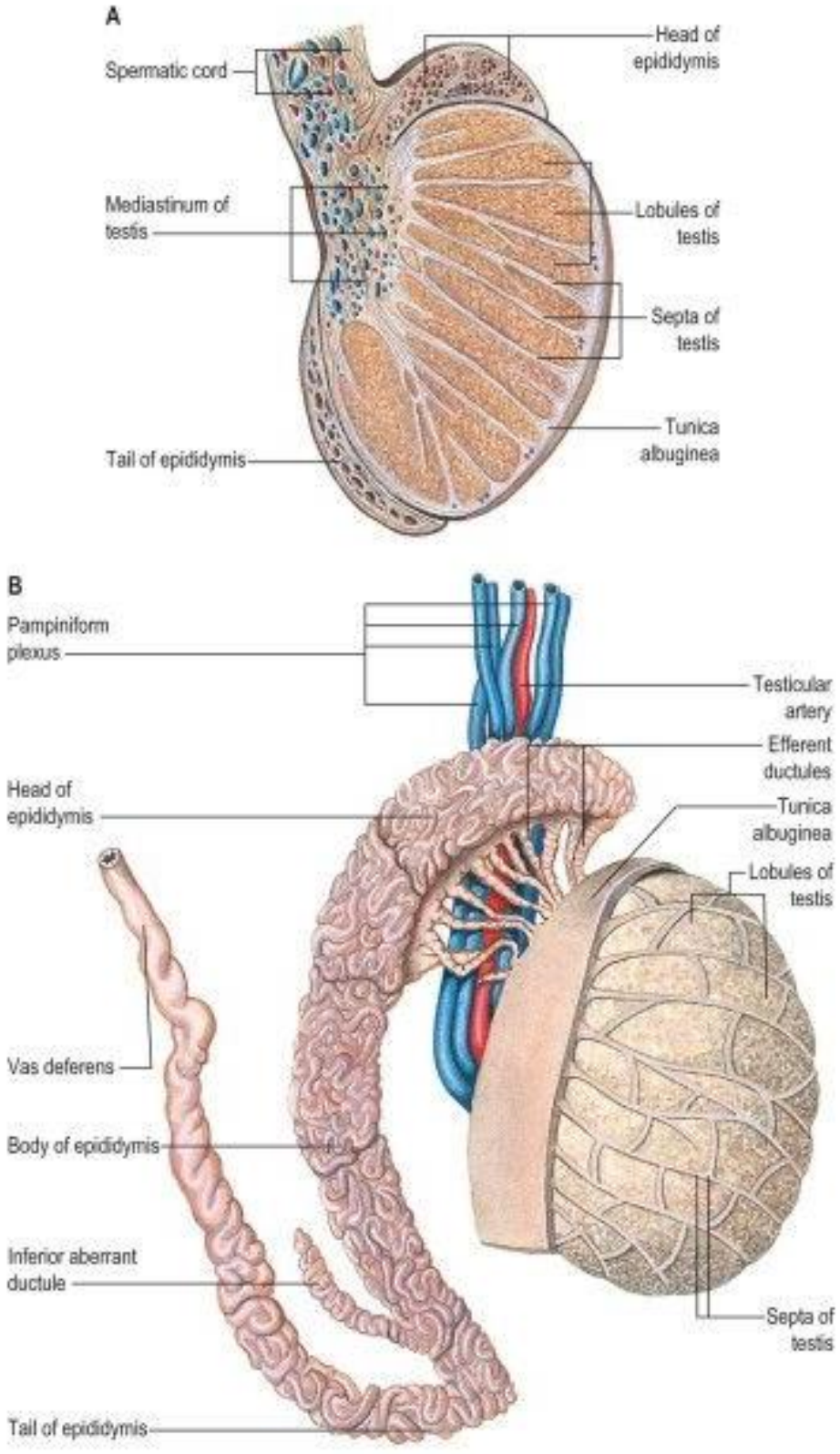
Each testis divided by multiple fibrous septae (The tunica albuginea, Mediastinum and fibrous septae connect to form the support and internal architecture of the testis) to form about 250-400 lobules, contain sperm-producing cells.

Each lobule drain sperms via a highly convoluted tubules "the seminiferous tubules".

These tubules converge on / enter Mediastinum testis to form > 20 larger ducts forming a network of channels within the stroma "the rete testis".

About 10-15 efferent ductules arise from superior Mediastinum & pierce the tunica albuginea to convey sperm to the head of the epididymis.

In about 90 % there is a small sessile projection "the testicular appendage "arises from the upper pole of the testis, just below the epididymis which represent a remnant of Mullerian duct (23).



Fig(3) internal structure of the testis (23).

1.2.3. Blood supply & heat regulation:

1.2.3.1. Arterial supply:

Each testis supplied by a long, slender testicular artery which originate directly from the aorta anteriorly at the level of renal arteries.

Each cross through retroperitoneal compartment & pass to the deep inguinal ring,

entering the spermatic cord & travel via the inguinal canal to enter the scrotum.
It divided multiple times & terminal branches either enter the testis over its surface or through Mediastinum testis.
Capillaries lying next to seminiferous tubules penetrate the layers of interstitial tissue and may form part of the 'blood–testis' barrier. They run either parallel to the tubules or across them but do not enter their walls. They are separated from germinal and supporting cells by a basement membrane and variable amounts of fibrous tissue containing interstitial cells(selective exchange phenomena involving androgens and immune substances)occur here, this also provide good heat regulation , & keeping testicular temperature less than rectal temperature by 2- 4 C.

The testis also receives blood from the cremasteric branch of the inferior epigastric artery, and from the artery to the vas deferens.
So interference with testicular artery high in the abdomen leaving the testis unharmed, while interruption in the spermatic cord may cause testicular infarction(23)

1.2.3.2. Venous drainage:
The testicular veins emerge posteriorly from the testis ,unite to form pampiniform plexus which drained by 3 -4 veins that run into the abdomen through the deep inguinal ring, these veins coalesce into two veins in the abdomen "the testicular veins" which ascend on each side of the testicular artery(23).
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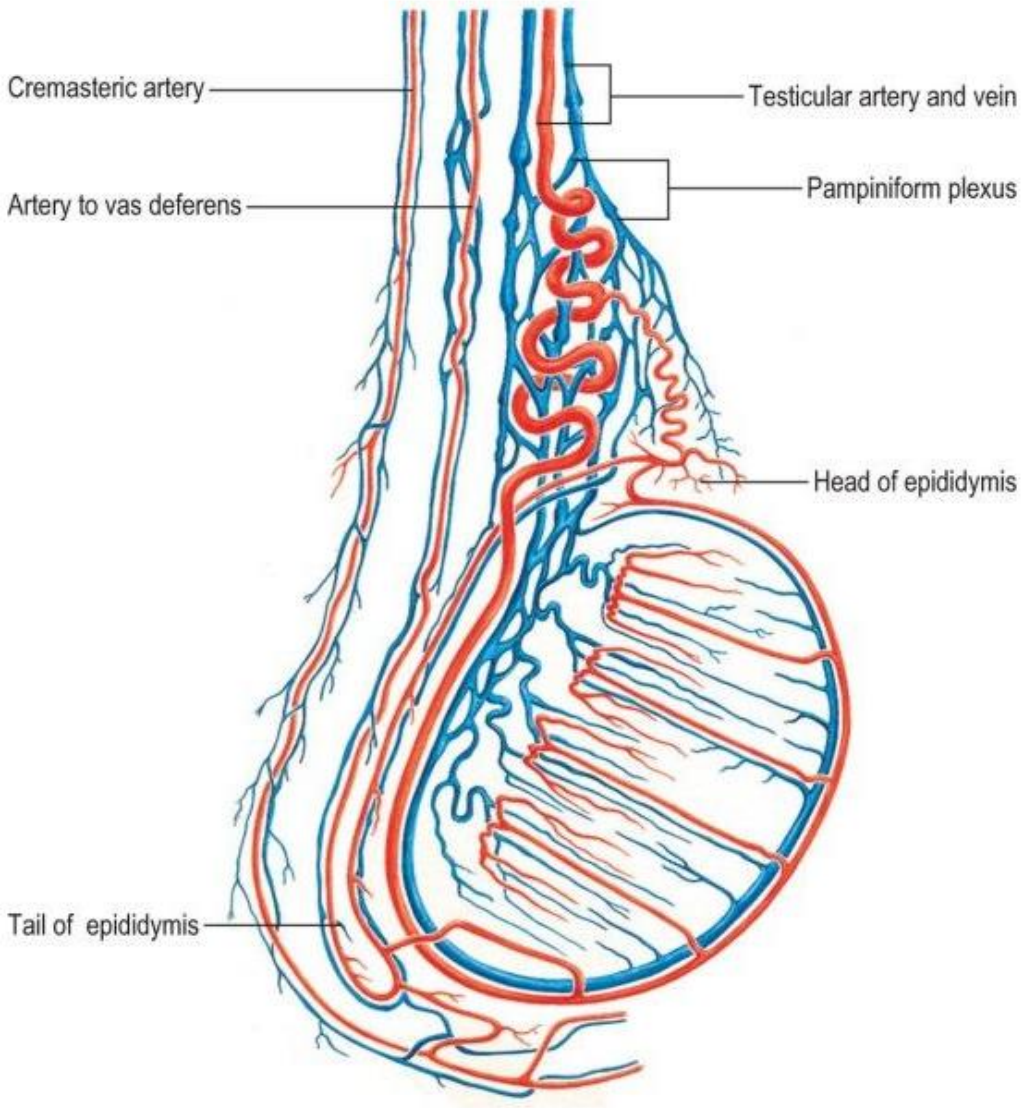


Fig (4). Arterial supply & venous drainage of the testis (23)

The right testicular vein opens into the inferior vena cava (IVC)at an acute angle just inferior to the level of the renal veins (RV), and the left testicular vein opens into the left renal vein at a right angle.
Thin wall, poorly muscularized venous system give it a somewhat unique characteristics (24).
Also and lack effective valves except at the inflow points into the IVC or the RV.
The higher frequency of varicocele on left (LT.) side may be explained in part by that;
The LISV drain to LRV in a right angle & the "nutcracker effect" by compression as it passes between the superior mesenteric artery & the aorta, both causes a higher intraluminal pressure& impaired flow through the LRV &LISV, especially in young men with limited retroperitoneal fat.
This will impair testicular temperature regulation & decrease intratesticular concentrations of testosterone & other local factors that important for spermatogenesis(24).

1.3. Elastography:
It is first suggested by Ophir et al (1991).
It is a developing technique that rises in the last two decades.
Tissue stiffness can be measured by using Young's modulus or elasticity (E).
E is obtained as the ratio between a uniform compression (i.e. Stress) and the resultant deformation (i.e. strain).
The tissue stiffness can then be converted and displayed as an image known as an Elastogram, which is usually color coded (25).
Young's modulus (E) = Stress(s) /Strain (e).
It is measured in Kilopascals (Kpa).
Hard tissues e.g. cancers will be hard on palpation & will reflect lower strain values& as a result will produce higher Young's modulus.(59).

1.3.1. Types of Elastography:
There are two types of elastography; Strain and Shear wave Elastography.
1.3.1.1. Strain Elastography (static /compression Elastography):
Usingan U/S probe, a gentle repeated compression is applied to the tissue under examination, resulting in tissue deformation (strain) which is measured by assessing the longitudinal movement of the tissue caused by the compression using radiofrequency (RF).
Elasticity of the tissue cannot estimated, it provide quantitative information only. Whoever a semi_ quantitative information on the nature of the tissue under examination can be gained by obtaining strain index, which is more accurate than quantitative information only (25).

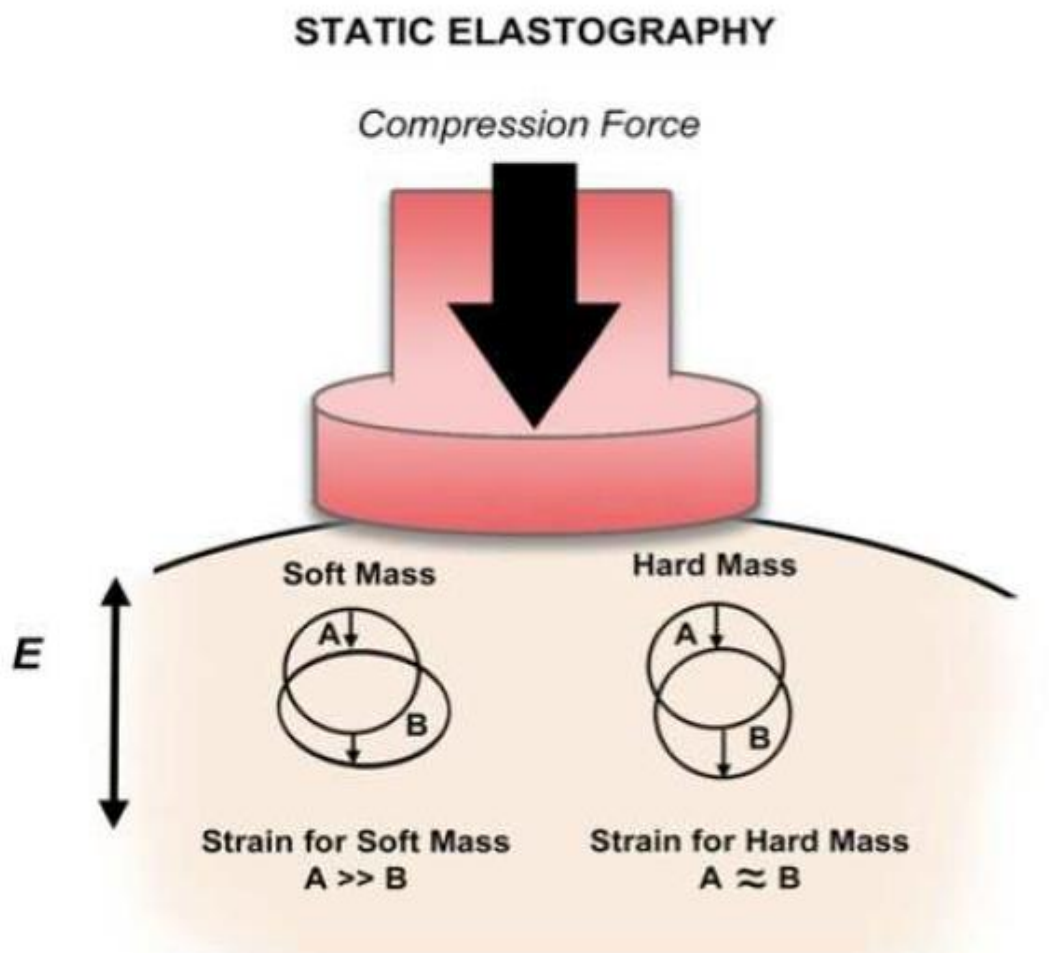


Fig (5). Strainelastography(25).

1.3.1.2. Shear Wave Elastography (transient elastography):

It uses an automated compression (in comparison to manual compression of strain elastography).

A transversely pulses from the U/S probe applied into the tissue under examination.

U/S probe will generate an automatic, gentle initial compression force pulses, introduced transversely into the tissue which will produce transversely oriented shear waves with in the tissue.

Stiffness (E) is directly proportion to the speed of the shear waves.

E=3PV2

E= elasticity.

P=density of the tissue.

V=velocity.

To obtain real time images, a very fast acquisition sequences were used.

The initial stress is readily to be quantified in Kpa, due its automated nature.

A hard tissue e.g. cancers will reflect high elasticity, because of that, the waves travel faster in hard tissues (25).

It is operator independent & reproducible in comparison with strain elastography.

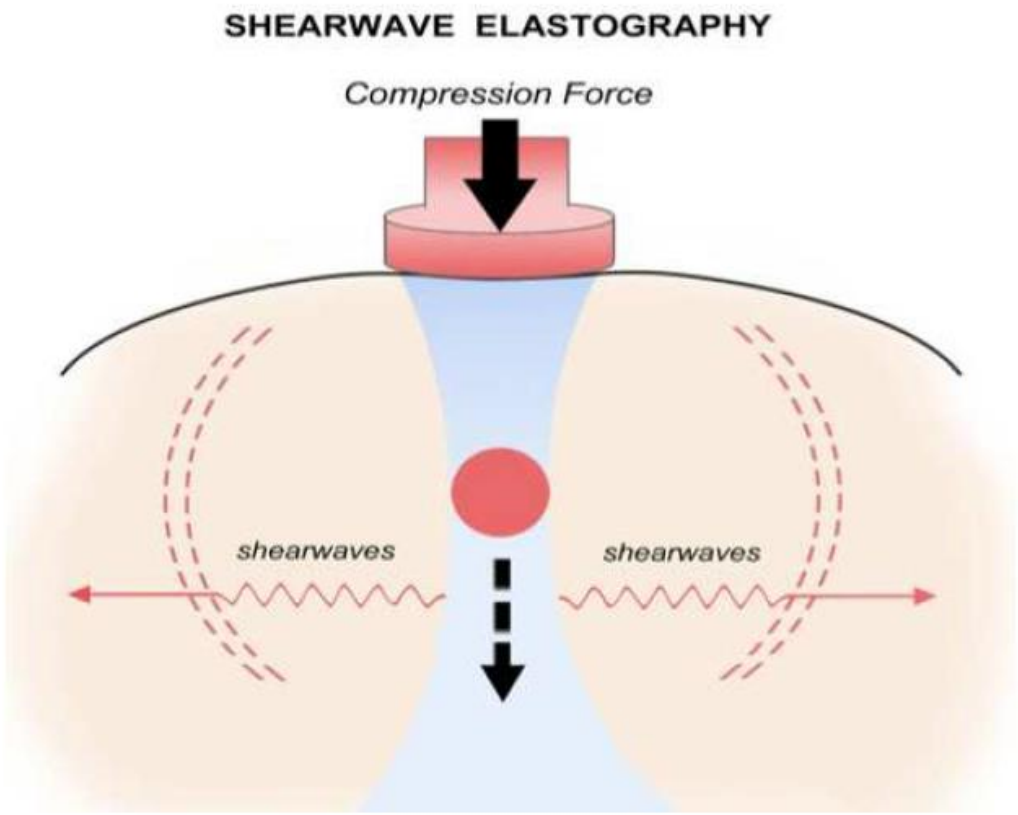


Fig (6). Shareware elastography(25).

Elastogram is the color coded image produced from strain or shear wave elastography that represent the tissue stiffness information, superimposed on a gray scale of the region of interest.

The coast; limit the availability of elastography equipped U/S machines (26 ,27 ,28).

Multiple researches contribute to infertility in male using sonoelastography& try to evaluate the internal testicular condition & some extend to differentiate between the obstructive & non-obstructive types using shear-wave& strain elastography with a cut off point for the strain ration of (0.36).

A colored map introduced with a gradient of red, yellow, green & blue colors represent a gradient from soft to hard testicle representation.

1.4. The aim of the study:

To evaluate the validity of sonoelastography in predicting positive sperm retrieving in patients with azoospermia.

2.1: patients and Methods:

A cross sectional study was conducted from 1st September 2017 to 1st September2018, at Al-Sadder teaching hospital / the fertility center & radiology unit.

(98) Patients with azoospermia proved by seminal fluid analysis (SFA) were evaluated & referred to the fertility center by urologist where prepared for testicular biopsy for diagnostic purposes or sperm harvesting.

After obtaining history and physical examination by specialists, the patients referred to do an U/S study in a form of B-mode, Doppler study & elastography (SR)…all patients with azoospermia were included in this study.

ES always performed before testicular biopsy to avoid altering the elasticity of the testis by invasive testing with bleeding or the start of scar formation.

Results of the biopsy were obtained by two embryologists with at least 5 years of experience in this field.

The results of the biopsy was presented by that "no sperms, 300K, 500K, 1M& 4M .... Etc. . . ."Where K & M represent number of sperms in thousands& millions respectively.

All these data were collected in a special form prepared for this purpose.

By using U/S machine (VOLUSONE6, GE Austrian) (figure 7 **)** which equipped by the property of sonoelastography by using the linear probe.

B-mode U/S was done to evaluate; testicular volume , echogeniety , associated mass , varicocele , hydrocele , hernia sac ,& any associated testicular or peri-testicular abnormality.

Doppler U/S performed to evaluate intra-testicular vascularity by evaluating the resistive index (RI) as in (fig 8)

Sonoelastography also done by calculating the SR.

This evaluation was performed during examination by the sonographers using the machine software.

The operators were asked to trace area A manually along the border of the testis. An area B was then selected just above the target testis as a reference, which was scrotal skin with a red ribbon.

Appropriate pressure was defined as pressure that could sustain the scale value at full / near full degrees (fig 9) .

The software calculated SR automatically.

Images were obtained from middle testicular portion & scrotal skin layer .as the ovoid shape of the testis, its poles couldn'tbe includedinto the image.

Each testis was assessed at least 3 times based on different static images and the average value was recorded as the final result.

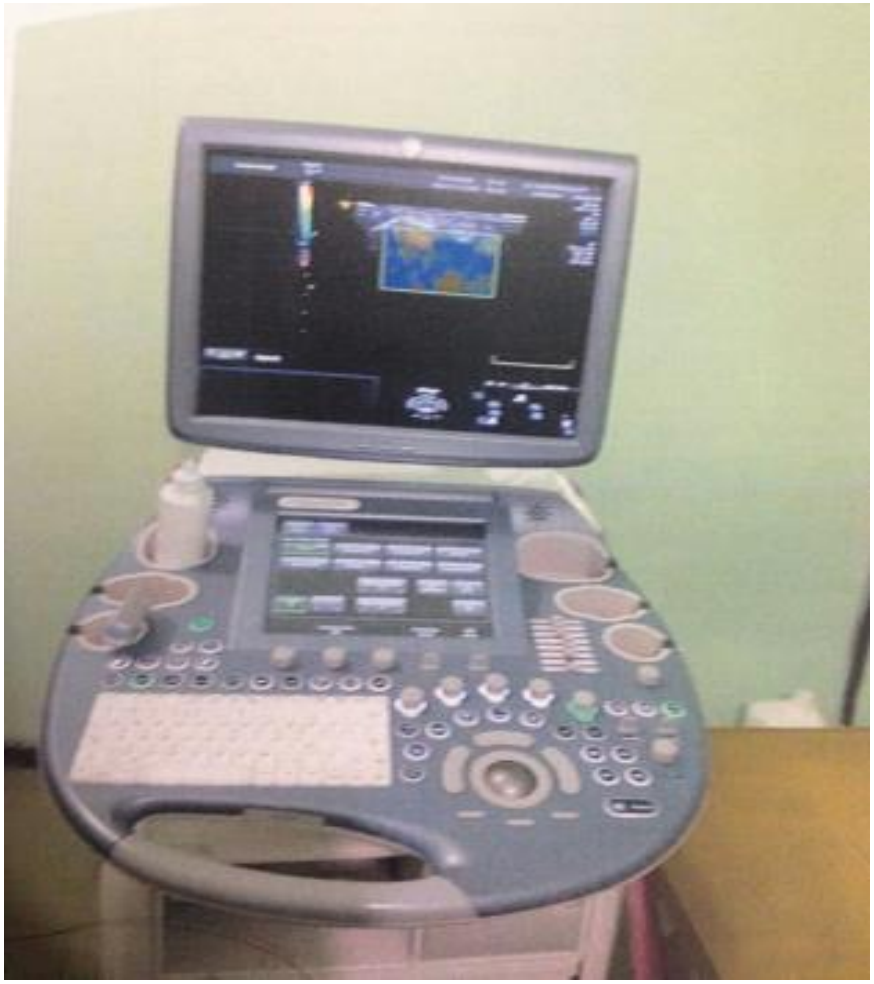


Fig (7).VOLUSONE6, GE Austrian U/S machine.

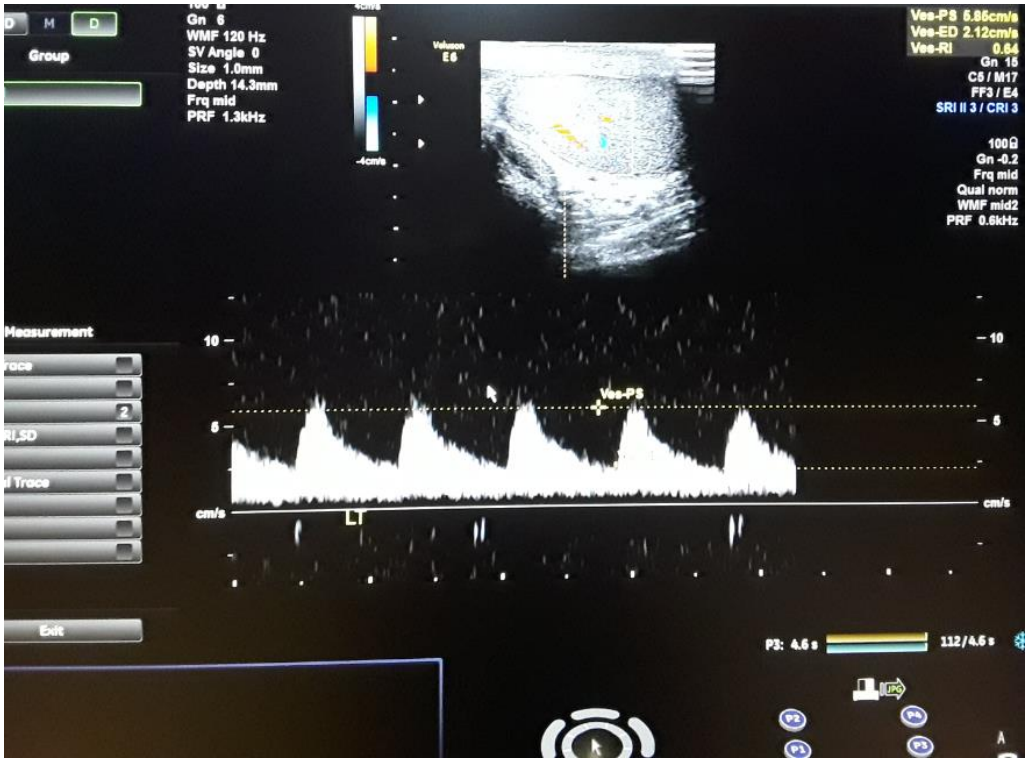
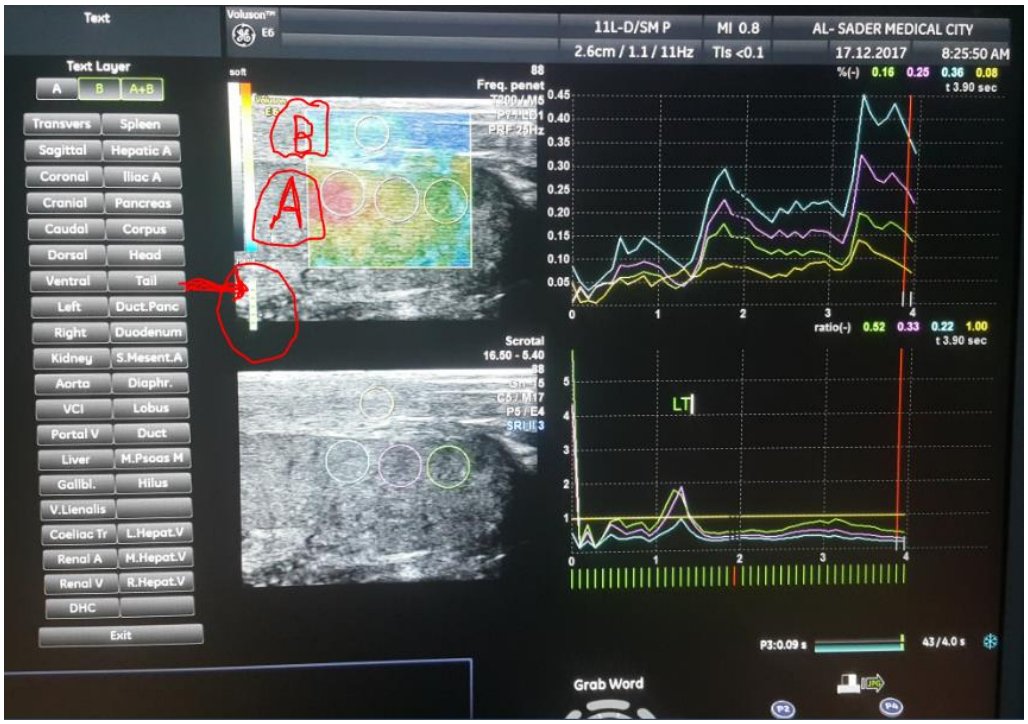


Fig (8). Doppler (RI) study.



Fig(9). Strain elastography to check strain ratio where :
(A) Represent different sites in the testicular parenchyma.
(B) Represent the scrotal skin as arefernce.
Red arrow scale value of appropriate pressure.

2.2: Equipment:

All examinations were done using an U/S machine GE version volusion E6 equipped with a 5 to 10 MHz linear probe have the elastography property.

Bilateral testicular volume were calculated in ml by using Lambert formula, length x height x depth x 0.7.

SR was performed by using two points A & B were they represent testicular elasticity & the reference (skin) respectively.

2.3: Patient preparations:

1- Verbal consent was taken from all patients before the U/S examination.

2- Explain the procedure to the patient.

3- Instruct the patient to keep still during the examination.

4- Use a suitable amounts of examination jell.

2.4: Positioning:

-Patient lie supine in a comfortable position.

-Area exposure with penis pulled over the abdominal wall.

-Covering of the unnecessary area.

-Make appropriate distance between the thighs.

-Patient asked to calm down as possible during examination.

2.5: Statistical analysis:

Statistical analysis was done using spss (statistical package for social sciences) version 20 numerical data expressed as mean +/-SD.

Categorical data expressed as frequencies and percentages.

Independent t- test for comparison of mean of 2 groups.

Chi square for comparison of categorical variable.

ROC curve for selecting cutoff for elastogram.

P value equal or less than 0.05 was regarded as significant

3.1. Results:

The total sample in this study was 98 patients, from them (36) were do a bilateral biopsy & the remaining (64) were do a uni-lateral biopsy.

Their mean age was 31.7±6.9 (with maximum of 60 and minimum of 20 (range =20), the other sociodemographic features had been shown in table (1)

Table 1: sociodemographic features of patients

Variable	Minimum	Maximum	Mean	SD
Age	20.00	60.00	31.7010	6.92544
Weight	61.00	96.00	79.9485	7.91935
Height	1.55	1.86	1.6853	.06136
BMI	18.79	34.84	28.1737	2.64566

Table 2: descriptive statistics for Doppler, volume and SR

Test	**Minimum**	**Maximum**	**Mean**	**Std. Deviation**
Doppler (RI)	**0.30**	**0.91**	**0.55**	**0.11**
Volume	**0.87**	**17.71**	**8.0321**	**3.37893**
SR	**0.18**	**2.73**	**0.7719**	**0.47417**

According to the biopsy the patients were divided into two groups : the first group was those with positive sperm retrieving(PSR)

(n=33) and the other group with negative sperm retrieving (NSR) (n=103). The mean strain ratio is significantly higher in those with NSR than PSR (0.89±0.04 vs 0.4424±0.1, p=0.0001*) while the mean

volume is significantly lower in those with NSR than PSR (8.25±0.4 vs10.4±0.5 ,p=0.002) as shown in figures (10) , (11),& table 3

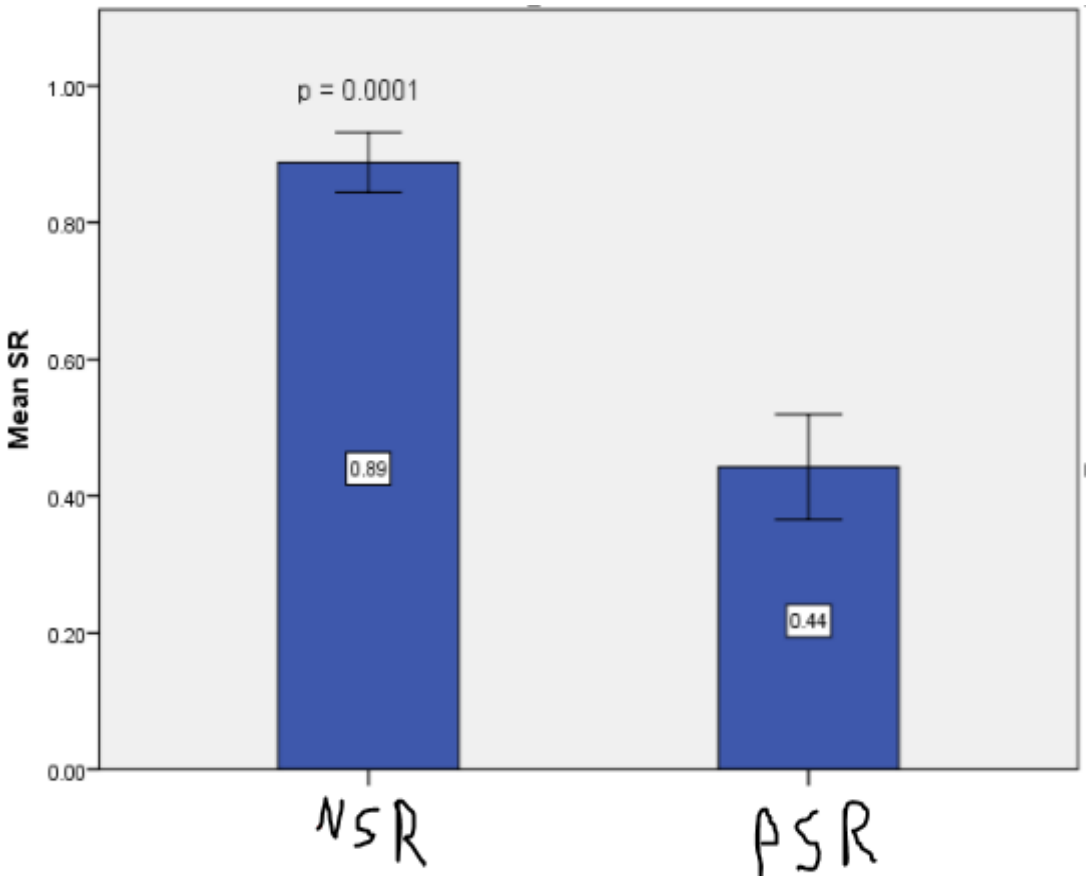


Fig (10)comparison of SR between PSR & NSR in azoospermic patients by mean -/+ SD.

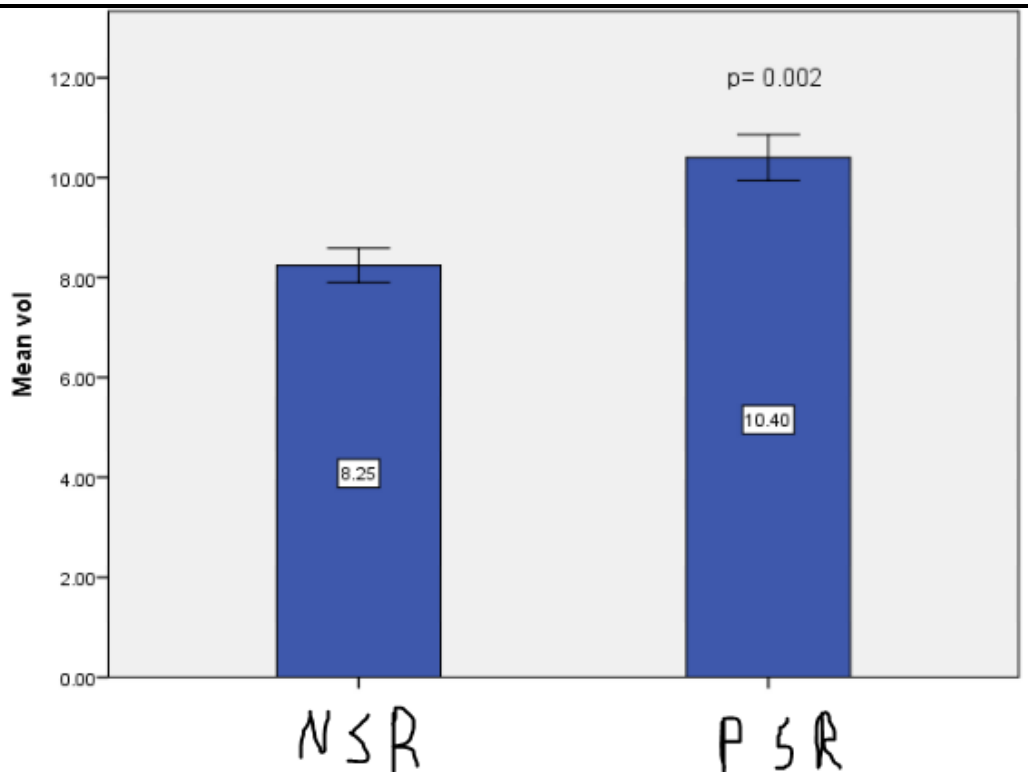


Fig (11) comparison of volume between NSR & PSR in azoospermic patients by mean -/+ SD.

Table 3: comparison of SR and testicular volume by mean±SE between NSR and PSR

	NSR	**PSR**	**P**
SR	**0.89±0.04**	**0.44±0.1**	**0.0001***
Volume	**8.25±0.4**	**10.4±0.5**	**0.002***

* Significant

There were positive correlation between volume and SR as r= 0.1 and p= 0.3) as shown in figure (3-3)

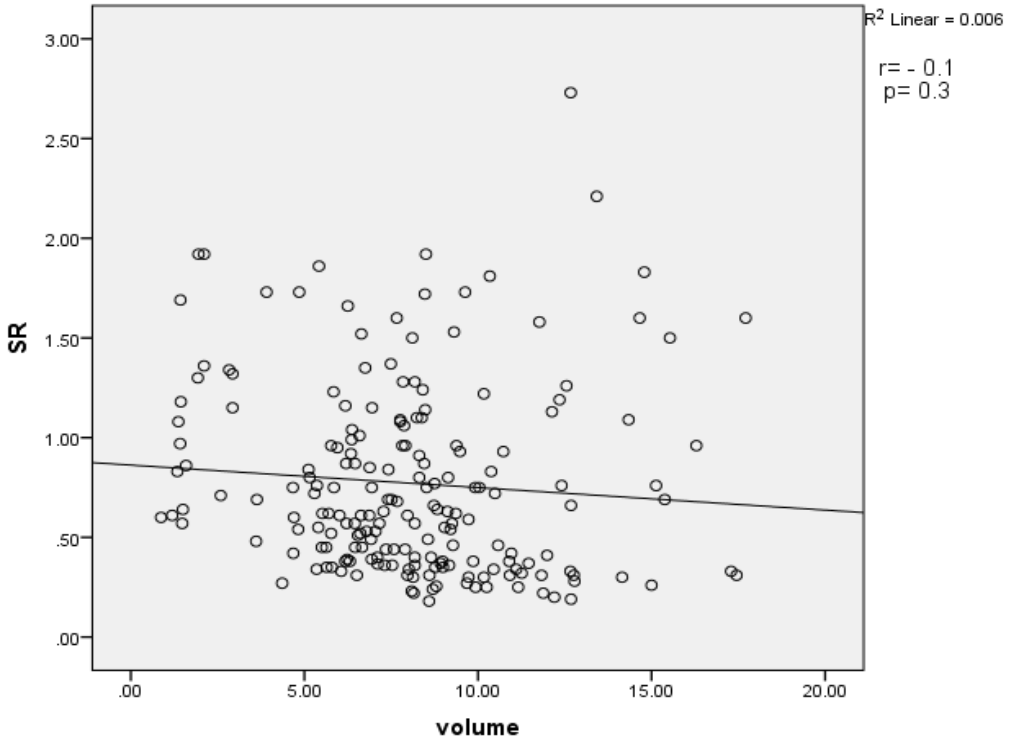


Fig.(12): correlation between testicular volume and SR .
High volume + low SR ➔ PSR ➔ good prognosis.
Low volume + high SR➔ NSR➔ bad prognosis.

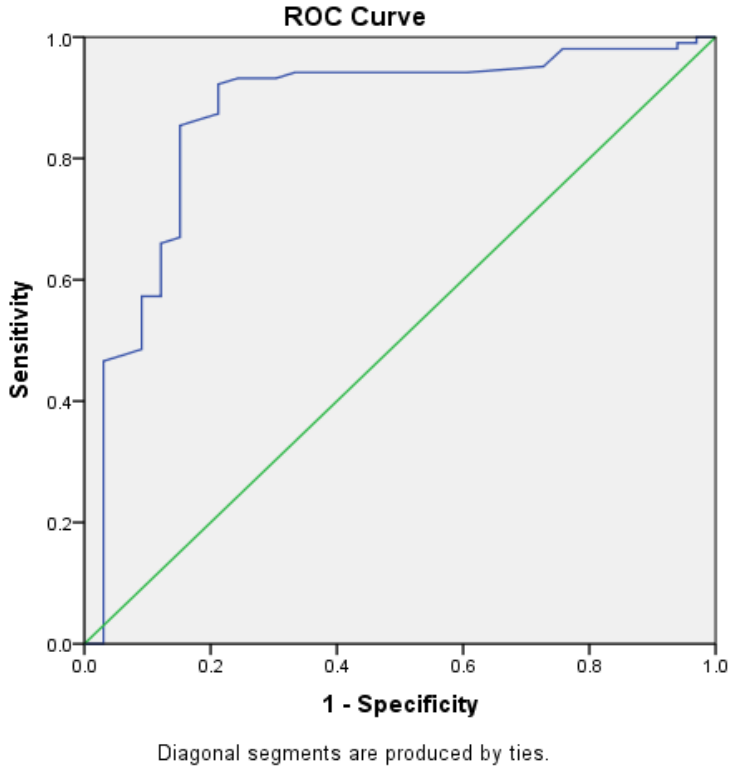


Fig.(13): ROC curve for strain ratio.
The area under curve was 0.871 which an indicator of an excellent test as shown in table 4.

Table (4): ROC test
Area Under the Curve

Test Result Variable(s): SR

Area	Std. Error	P value	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
0.871	0.040	0.0001	0.792	0.950

Table 5: validity of strain ratio in differentiation between PSR and NSR (Cutoff value = 0.385)

		Biopsy		**Total**	**P**
		PSR	NSR		
SR	0.385	25 (75.8%)	7 (6.8%)	32 (23.5%)	0.0001
	≥0.385	8 (24.2%)	96(93.2%)	104(76.5%)	
Total		33(100%)	103(100%)	136 (100%)	

Regarding validity of strain ratio in differentiation between PSR and NSR 104 patients had SR of 0.385 and above, 96 of them proved by biopsy. Of the total 32 patients with SR 0.385, only 7 were proved by biopsy to have NSR.
These findings of SR gives a
Sensitivity of 75.5%
Specificity= 93.2%
Positive predictive value= 78%
Negative predictive value= 92.3 and
Accuracy of 89%.

Discussion

Infertility is a common worldwide public health affair as that more than 15 % of couples may be suffer from & the subsequent psychosocial problems may impair partner relationships.
Nowadays imaging modalities are more commonly used to evaluate the causes of infertility , of these modalities , the ordinary (B-mode ,&color Doppler)U/S with its recently equipped techniques(elastography with SR & SWE) were the first line in investigating male infertility & to evaluate testicular tissue & spermatogenesis.(29 ,30).
To be known that" this is the first study to evaluate the SR for diagnosing azoospermia in our teaching city.
Strain elastography is based on the criteria of that compression Produces strain within the tissue and the amount of strain is lower in stiffer tissue than in softer. (31).
Some focal testicular lesions like microlithiasis, azoospermia, & small lesions of < 10 mm diameter, especially if not palpable, were investigated by ES.(32 ,33).
The biopsy results of azoospermic male patients showed that along with the increasing grade of histological criteria , seminiferous tubules diameter & spermatogenic epithelium height are gradually decreased but the lamina properia thickness was gradually increased.(32)
Authors concluded that tissue stiffness was increased in azoospermic patients and SR may be useful for diagnosing azoospermia.

SR was proved by some studies to provide more objective data with a diagnostic accuracy more than elastography.(33,34,35).

Further studies evaluating the relationship between SR results and testicular sperm retrieval rates are needed to increase the diagnostic accuracy of this imaging modality. Our study revealed that SR values were significantly higher in patients with abnormal semen parameters.

Many studies hypothesized that testicular volume may be a foreteller of spermatogenic function. Tijani et al. (36) reported that testis volume was significantly different in fertile and infertile groups.

Further studies revealed that volume had a significant correlation with semen volume, sperm count & motility. (37, 38)

Condorelli et al. (39), reported that testis volume was associated with some biofunctional sperm parameters and stated that the biofunctional sperm parameters worsen with decreasing testicular volume.

Schurich et al. (40) reported that elastography can be used for structural analysis of testicular tissues in order to find out any pathological tissue alterations.

Our study also show that mean volume is significantly lower in those with non-obstructive azoospermia than obstructive azoospermia.

Since seminiferous tubules which constitute 80% of testicular volume were responsible for spermatogenesis, testicular volume can be related to sperm count.

Using ES we can discriminate soft from hard testicular tissue regions, this based on that a backscattered sonographic signals suffer from displacement if the tissue is slightly compressed &decompressed.

Lesser displacement seen when tissue get stiffer than normal tissue.

So, tissue elasticity may be correlated with pathological conditions

5.1. Conclusion:

Strain elastography results were found to be significantly different in patients with abnormal sperm counts. This technique may provide promising results, however, further large scale studies may help to clarify the value of this imaging modality in the assessment of male infertility.

5.2. Recommendations;

1) Large studies should be carried out to discover the beneficial applications of this modality with inclusion of hormonal status of the patients.

2) by using elasticity score (ES) we can evaluate the intr-testicular elasticity & the more elastic area(more chance of focal spermatogenesis) according to the color scale to be a good guide for the sperm collection before the biopsy.

5.3. Limitations:

According to most researches a correlation of hormonal state of the patients is an important issue to differentiate the OA from NOA, but regrettably most of our patients didn't make a hormonal survey before the biopsy.

References

1. Jarow JP, Espeland MA, Lipshultz LI. Evaluation of the azoospermic patient. J Urol. 1989;142(1):62-5.
2. WHO. World Health Organization: WHO Laboratory manual for the examination and processing of human semen - 5th ed. Geneva: WHO Press, 2010.
3. Schlegel PN. Causes of azoospermia and their management. Reprod Fertil Dev. 2004;16(5):561-72, http://dx.doi.org/10.1071/RD03087.
4. The epidemiology and etiology of azoospermia Marcello Cocuzza, ConradoAlvarenga, Rodrigo Pagani
5. Matsumoto AM.. Pathophysiology of male infertility. In: KeyeWR,Chang RJ, Rebar RW, Soules MR. Infertility evaluation and treatment. USA: W.B.Saunderscompany. 1995; P 555-579.
6. Matsumoto AM.. Pathophysiology of male infertility. In: KeyeWR, Chang RJ, Rebar RW, Soules MR. Infertility evaluation and treatment. USA: W.B.Saunderscompany. 1995; P 555-579.
7. RowePJ, ComhaireFH, Hargreave TB, Mahmoud AM. WHO manual for the standardized investigation and

diagnosis of the infertile male. UK: Cambridge University press; 2000. p91.
8. Esteves SC, Oliveira FV, Bertolla RP. Clinical outcome of intracytoplasmic sperm injection in infertile men with treated and untreated clinical varicocele. J Urol. 2010;184(4):1442-6.
9. Agarwal A, Deepinder F, Cocuzza M, Agarwal R, Short RA, Sabanegh E, et al. Efficacy of varicocelectomy in improving semen parameters:new meta-analytical approach. Urology. 2007;70(3):532-8, http://dx.doi.org/10.1016/j.urology.2007.04.011.
10. MarmarJL, Agarwal A, PrabakaranS,Agarwal R, Short RA, BenoffS,et al. Reassessing the value of varicocelectomy as a treatment for male subfertility with a new meta-analysis. FertilSteril. 2007.
11. MathersMJ, Sperling H, Rubben H, Roth S. The undescended testis: diagnosis, treatment and long-term consequences. DtschArztebl Int.2009;106(33):527-32.
12. Grasso M, Buonaguidi A, Lania C, Bergamaschi F, Castelli M, Rigatti P.Postpubertal cryptorchidism: review and evaluation of the fertility. EurUrol. 1991;20(2):126-8.
13. Williamson RC. Torsion of the testis and allied conditions. Br J Surg.1976;63(6):465-76.
14. Watkin NA, Reiger NA, Moisey CU. Is the conservative managementof the acute scrotum justified on clinical grounds? Br J Urol.1996;78(4):623-7.
15. Davis NF, McGuire BB, Mahon JA, Smyth AE, O'Malley KJ, FitzpatrickJM. The increasing incidence of mumps orchitis: a comprehensivereview. BJU Int. 2010;105(8):1060-5, http://dx.doi.org/10.1111/j.1464-410X.2009.09148.x.
16. Werner CA. Mumps orchitis and testicular atrophy; a factor in malesterility. Ann Intern Med. 1950;32(6):1075-86.
17. Werner CA. Mumps orchitis and testicular atrophy; occurrence. AnnIntern Med. 1950;32(6):1066-74.The Epidemiology and Etiology of Azoospermia Cocuzza M et al.CLINICS 2013;68(S1):15-2624
18. NudellDM, Monoski MM, Lipshultz LI. Common medications anddrugs: how they affect male fertility. UrolClin North Am.
19. 2002;29(4):965-73, http://dx.doi.org/10.1016/S0094-0143(02)00079-4.
20. The management of infertility due to obstructive azoospermia. Fertil Steril. 2008;90(5 Suppl):S121-4.
21. Ferlin A, Raicu F, Gatta V, Zuccarello D, Palka G, Foresta C. Male infertility: role of genetic background. Reprod Biomed Online.2007;14(6):734-45, http://dx.doi.org/10.1016/S1472-6483(10)60677-3.
22. Matsuda T, Horii Y, Yoshida O. Unilateral obstruction of the vas deferens caused by childhood inguinal herniorrhaphy in male infertility patients. FertilSteril. 1992;58(3):609-13.
23. Handelsman DJ, Conway AJ, Boylan LM, Turtle JR. Young's syndrome.Obstructive azoospermia and chronic sinopulmonary infections.N Engl J Med. 1984;310(1):3-9, http://dx.doi.org/10.1056/NEJM198401053100102.grant;s atlas of anatomy twelfth edition.
24. Reuter, VE. Anatomy and Pathology of Testis Cancer. Scardino PT, LinehamWM, ZelefskyMJ&Vogelzang NJ (eds.). (2011). Comprehensive Textbook of Genitourinary Oncology. (4th Edition). Philadelphia: Wolters Kluwer/Lippincott Williams & Wilkins. 31:531-543. Retrieved from: http://solution.lww.com.
25. Institute for advanced medical education (online CME) Breast elastography.
26. Yurong Hong, Xueming Liu, Zhiyu Li et al. Real time ultrasound elastography in thedifferential diagnosis of benign and malignant thyroid nodules. Journal of ultrasound inmedicine: July 2009 vol28 no 7: p 861-867.

27. Honea KL. Understanding unexplained infertility. In: Carr BR,Blackwell RE. Reproductive medicine. USA: Appleton and Lange.1993; P 537-545.
28. Forman R, Gilmour-White S, Forman N. Recreational drugs and drugs of abuse. In: Drug-induced infertility and sexual dysfunction. UK:Cambridge University press. P 106-123.
29. Arslan H, Sakarya ME, Atilla MK. Clinical value of powerDoppler sonography in the diagnosis of varicocele. J ClinUltrasound 1998;26:229. [CrossRef]
30. Zhang X, Lv F, Tang J. Shear wave elastography (SWE) is reliablemethod for testicular spermatogenesis evaluation after torsion. IntJ ClinExp Med 2015;8:7089-97.
31. Ophir J, Kallel F, Varghese T, Alam SK, Krouskop T, Garra BS,et al. Elastography. Optical and Acoustical Imaging of Biological Media 2001;4:1193-212.
32. Li M, Du J, Wang ZQ, Li FH. The value of sonoelastography scores and the strain ratio in differential diagnosis of azoospermia.
33. J Urol 2012;188:1861-6.
34. Pastore AL, Palleschi G, Maceroni P, Manfredonia G, Autieri D,Cacciotti J, et al. Correlation between semiquantitativesonoelastographyand immunohistochemistry in theevaluation of testicularfocal lesions. Cancer Imaging 2014;14:29..
35. Zhi H, Xiao XY, Yang HY, Wen YL, Ou B, Luo BM, et al. Semiquantitatingstiffness of breast solid lesions in ultrasonic elastography.AcadRadiol 2008;15:1347-53. [CrossRef]
36. Thomas A, Degenhardt F, Farrokh A, Wojcinski S, Slowinski T,Fischer T. Significant differentiation of focal breast lesions: calculationof strain ratio in breast sonoelastography. AcadRadiol2010;17:558-63. [CrossRef].
37. Stein RJ, Santos S, Nagatomi J, Hayashi Y, Minnery BS, XavierM, et al. Cool (TRPM8) and hot (TRPV1) receptors in the bladderand male genital tract. J Urol 2004;172:1175-8.
38. TijaniKH, Oyende BO, Awosanya GO, OjewolaRW, Yusuf AO.Assessment of testicular volume: A comparison of fertile andsub-fertile West African men. African J Urol 2014;20:136-40.
39. Kumar S, Mohsen N, VineethVS, Malini SS. Assessment ofTesticular Volume in Correlation with Spermiogram of InfertileMales in South India. Advanced Studies in Biology 2013;5:327-[CrossRef]
40. Kristo A, Dani E. The Correlation between Ultrasound Testicular Volumeand Conventional Semen Parameters in Albanian Subfertile Males.Macedonian Journal of Medical Sciences 2014;7:464-6.
41. Condorelli R, Calogero AE, La Vignera S. Relationship betweentesticular volume and conventional or nonconventional sperm parameters. Int J Endocrinol 2013;2013:145792.