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Effect of grading of varicocele on sperm parameters, oxidative stress and Chromatin maturityand inhibin B levels of infertile patients with varicocele

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Abstract:

The current study included 78 infertile patients complaining from varicocele with three different grading (garde1:n=42, grade2:n=23, grade3:n=13)who attended to fertility Center in Al-Sadr Medical City in Al-Najaf Health Directorate/Ministry of Health/Iraq.This study aimed to determine the effect of grading of varicocele on sperm parameters and the levels of MDA (Malondialdehyde), ROS (Reactive oxygen species), GSH (Glutathione), SOD1 (superoxide dismutase1), CAT (Catalase) in semen, Inhibin B concentration in semen and serum alsosperm chromatin maturity percent in seminal fluidof infertile patients with varicocele.

The result showed the sperm parameters quality, GSH,SOD1,CAT, Inhibin B levels and sperm chromatin maturity percent in infertile with varicocele grade 3 were significant decreased($P < 0.05$) compared to infertile with varicocele grade 2, also the MDA and ROS concentration revealed significant increase in the same comparison.It was concluded:the severity of varicocele can lead to a significant harmful effect on sperm parameters,oxidative stress, chromatin maturity percent and level of inhibin B in serum and seminal plasma of infertile male who complaining of varicocele.

Key words: Varicocele, grading of Varicocele, seminal fluid,MDA, ROS, GSH,SOD1,CAT, Inhibin B,chromatin maturitypercent.

Introduction:

The varicocele is an abnormal dilatation and tortuosity of veins of the pampiniform plexus that drain the testis within the spermatic cord (Rajeev andRupin 2005).Varicoceles are present in 15% of the normal general male population and in approximately 40% of infertile male which cause testicular temperature elevation and venous reflux appear to play an important role in varicoceleinduced testicular dysfunction(Nagleret al., 1997). Impairment of testicular microcirculation has been proposed as part of the pathologic effects of varicoceles (Sweeney et al., 1995)

The term of varicocele refer to those recognizable with palpation or visual inspection. Dubin grading system is the most widely used classification "Dubinand Amelar, 1970"(Pauroso, *et al.*, 2011)Grade 1: palpable through valsalva maneuver examination.Grade 2: palpable at rest condition.Grade 3: visible and palpable at rest condition.

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Oxidative stress is a result of an imbalance between reactive oxygen species (ROS) production and the antioxidant mechanism defense against them (Agarwal and Prabakaran, 2005). Nowadays many investigations showed strong relation between oxidative stress and male infertility with varicocele (Ishikawa et al., 2007; Smith et al., 2006) also there are some hormonal factor play an important role in male infertility such as inhibin B which secret in response to FSH by sertoli cells in males (Ying, 1988).Present study aimed to determine the effect of varicocele grading onsperm parameters and the levels of MDA(Malondialdehyde), ROS (Reactive oxygen species), GSH (Glutathione), SOD1 (superoxide dismutase1), CAT (Catalase) in semen, Inhibin B concentration in semen and serum also chromatin maturity percent in seminal fluidof infertile patients with varicocele.

Material and methods:

The current study included 78 infertile patients complaining from varicocele. The clinical assessment was evaluated by a specialist urologist for detecting varicocele, hydrocele, cryptorchidism, hernia and other congenital abnormalities in adding using testicular ultrasound (sonogram), then seminal fluid analysis was performed according to WHO (World Health Organization) (1999) guidelinesto determine the sperm parameters include:

Sperm concentration (million/ml): Sperm concentration was calculated through the mean number of sperms in 10 microscopically fields randomly and the mean number of sperms multiply by a factor of one million (106) and total sperm count was obtained by multiplying sperm concentration by semen volume.

Progressive sperm motility percent: The progressive motile sperm was counted by taking the mean number of forward progressive motile spermatozoa.

Normal morphology percent: normal sperm morphology was reported according the following equation: Normal sperm morphology = No. of normal sperm / Total sperm count X 100.

Evaluation of chromatin maturity: in the current study used aniline blue staining method for evaluation of chromatin maturity percent of sperms.The Slide was prepared by smearing 5 µl of seminal samples. The slides were dried in air and maintained 30 minutes in glutaraldehyde (3%) (Diluted in phosphate buffer saline (PBS)) for fixation. Slides were dehydrated then smears stained in aqueous aniline blue solution 5% (dissolved 5g of aniline blue per 100 ml of PBS) (pH 3.5) for 5 minutes. The heads of Sperms which contain immature nuclear chromatin seen as blue color, but sperms with mature nuclear chromatin did not stain and remained colorless(fig.1). The spermatozoal proportion which stained with aniline blue is measured by counting two hundred sperms in each slide by light field microscope (Hammadeh*et al*., 2001).

Biochemical assay:the levels of Glutathione (GSH) , superoxide dismutase1 (SOD1) and Catalase (CAT) measured by Elabscience's assay kit and for evaluation of Reactive oxygen species (ROS) and Inhibin B were used Creative diagnostic's assay kit which determined by Elisa apparatus while the Malondialdehyde (MDA) concentration measured by modified procedure described by "Guidet and Shah, 1989" (Raad*et al.*, 2002).

Results:

The current results showed significant decline($P<0.05$) in sperm concentrations, progressive sperm motility percent and normal morphology percent in infertile men complaining of varicocele

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grad 3 compared to infertile groups with varicocele grade 2 and grade 1, While there were non-significant difference (P>0.05)of mention parametersbetween grade 1 and grade 2 (Table 1).

The current results revealed significant increase(P < 0.05) in seminal ROS level of infertile with varicocele garde 3 group compared to grade1 and grade2, while there wasn't significant differences(P>0.05) between grade 2 & grade1 . The MDA concentration showed a significantly increase (P<0.05) in varicocele grade 3 compared to grade 1 and grade2 also there were significant elevation in MDA levels (P<0.05) of varicocele grade2 when compared with the MDA level of varicocele grade1.

Seminal plasma GSH and SOD1 were significant decrease (P < 0.05) of varicocele grade 3 compared to grade 1 and grade2, also there was significantly decrease (P < 0.05) when compared to levels of those parameters in varicocele grade2 with grade1. While there was just significant decrease of CAT levels (P < 0.05) in varicocele grade3 compared to varicocele grade2 and non-significant differences (P > 0.05)in CAT concentration among grade 2 & grade1 (Table 2).

Also the results of this study noticed a significant decline (P<0.05) of chromatin maturity percent in varicocele grade 3 compared to grade 1 and grade2 and there was a significantly decrease (P < 0.05) when levels of this factor in varicocele grade2 compared to varicocele grade1 (fig.2).

The results of this study showed a significantly decline (P<0.05) in sera and seminal Inhibin B levels of infertile men complaining of varicocele grad 3 compared to serum and seminal plasma of grade 1 and grade 2 while decreasing of inhibin B concentration in grade 2 specimens compare to infertile men affected by varicocele grad1 not significant (P > 0.05)(fig.3 and 4).

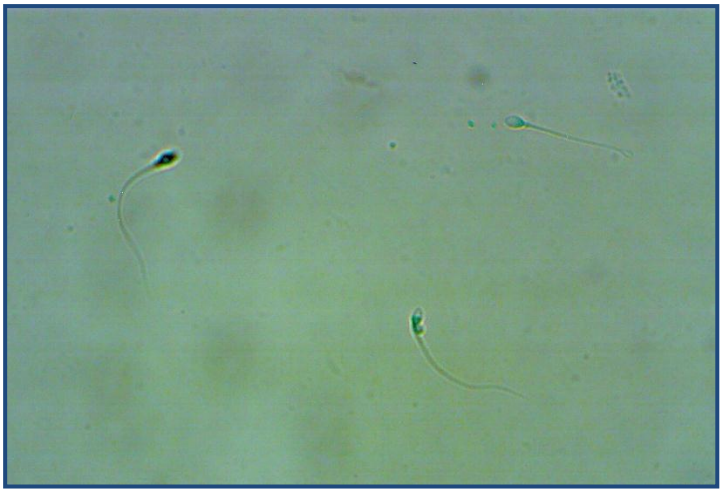

Fig. (1): Sperm Cells stained by Aniline Blue Staining A: Sperm with unstained head (Mature Chromatin) B: Sperm with blue head (Immature Chromatin)

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Table 1: The effect of grading of varicocele on semen parameters in infertile patients.

Groups / Seminal parameters	Grad 1 N=42 mean ± SD	Grad 2 N=23 mean ± SD	Grad 3 N=13 mean ± SD
Sperm Concentration (million/ml)	36.78±27.80 (a)	34.51±26.28 (a)	18.28±14.46 (b)
Progressive motile sperm percent (%)	45.28±28.23 (a)	42.50±32.13 (a)	39.05±21.56 (b)
normal Morphology (%)	56.53±2.40 (a)	55.75±5.92 (a)	53.75±7.47 (b)

*Various letters revealed significant differences (P < 0.05) between means.

Table2 : the effect of grading of varicocele on antioxidant and oxidant levels and DNA integrity.

Groups / parameters	Grad 1 N=42 mean ± SD	Grad 2 N=23 mean ± SD	Grad 3 N=13 mean ± SD
ROS (ng/ml)	0.53 ±0.23 (a)	0.5 ±0.19 (a)	0.97 ± 0.25 (b)
MDA (um/l)	6.03 ± 1.69 (a)	9.71± 1.97 (b)	13.76 ±2.82 (c)
GSH (ug/ml)	2.14 ± 0.11 (a)	2.02 ±0.15 (b)	1.88 ±0.12 (c)
SOD1 (pg/ml)	301.67± 13.2(a)	249.92± 11.6(b)	235.255 ±11.4 (c)
CAT(pg/ml)	5.2 ± 0.31(a)	4.77± 0.38(a)	3.12± 0.24 (b)

*Various letters revealed significant differences (P < 0.05) between means

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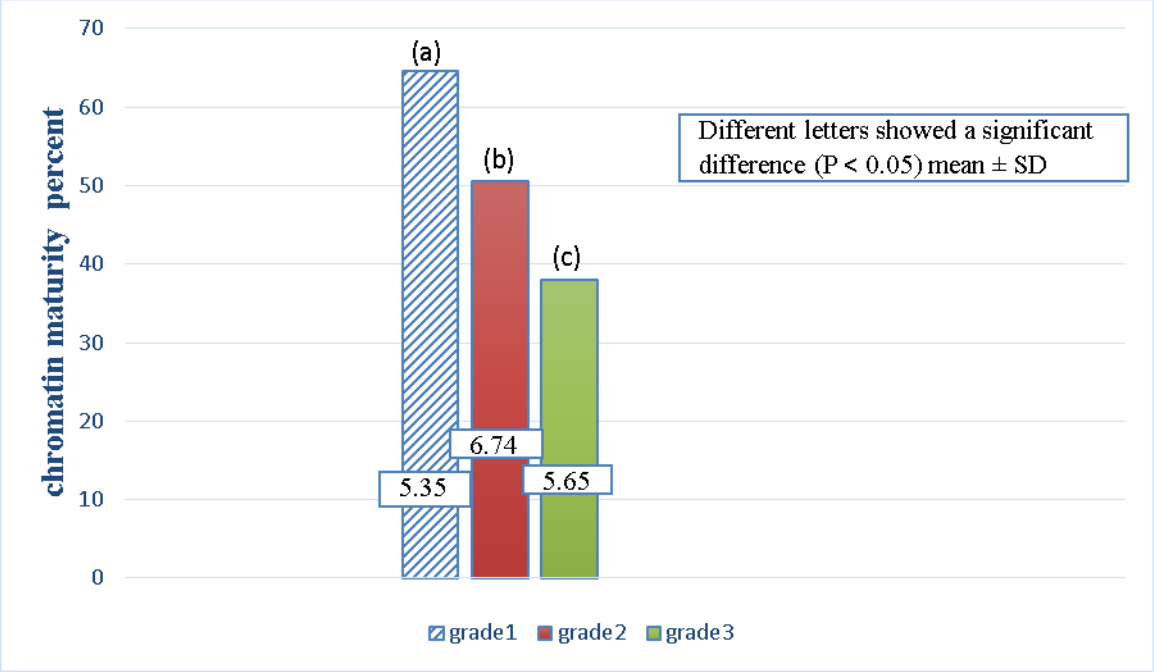

Figure 1 :The effect of grading of varicocele on chromatin maturity

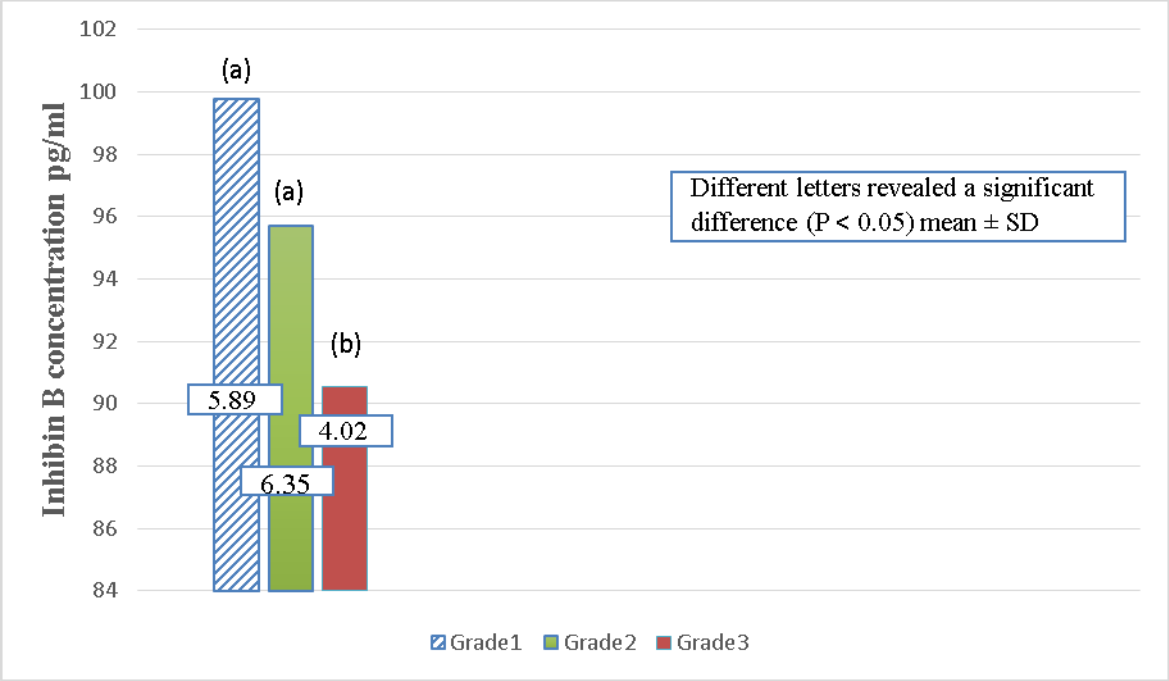

Fig.2 :Effect of grading of varicocele on inhibin B levels in serum of infertile male with varicocele.

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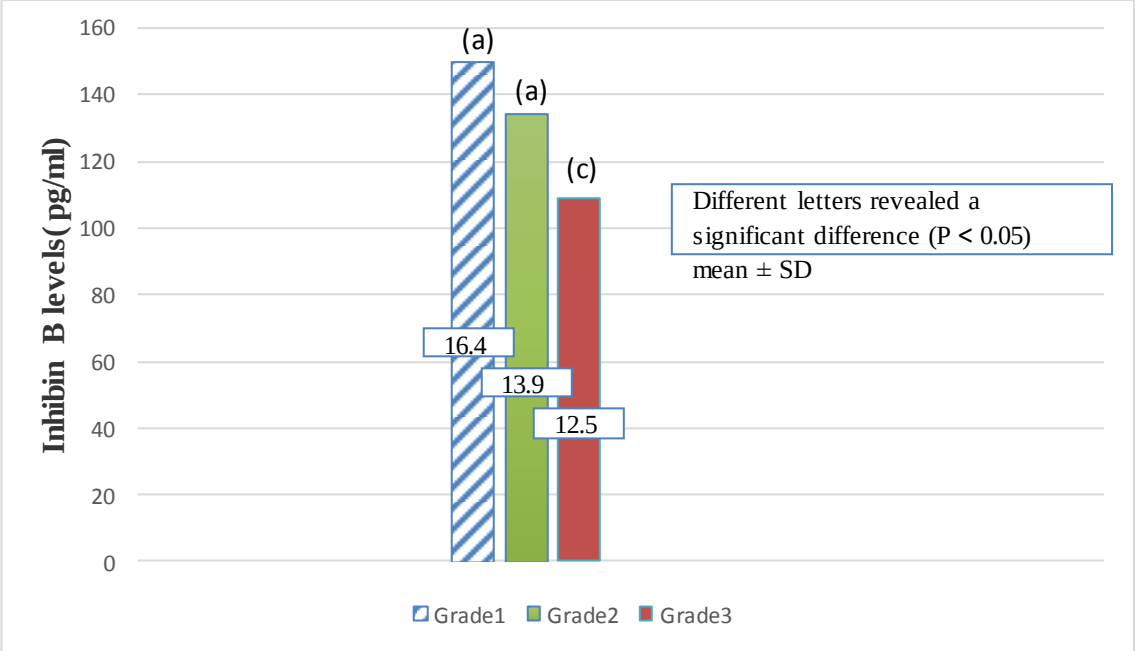

Fig.3:Effect of grading of varicocele on inhibin B levels of seminal plasma in infertile male with varicocele.

Discussion:

The severity of the varicocele caused significant reduction (P <0.05) in the sperm concentration , progressive motile sperm percent and normal sperm morphology percent for patients with varicocele Grade3 compared with patients with varicocele Grade1 and Grade2, this result may be due to increase the temperature about 2.5 C^{o} with increasing the severity of the injury, which caused a decline of blood flow to the scrotum, which lead to reduced oxygenation of the testis that leads to reduce the maturation of sperm and a reduction of ATP and increase nitric oxide, which affects testicular germ cells (Cocuzza*et al*., 2007; Kothari *et al*.2010). This mention in previous studies which revealed that an inverse relationship between the severity of the injury and the sperm parameters of patients with varicocele (Ishikawa and Fujisawa, 2005;Shamsa, 2007). There are a relationship between the severity of the varicocele with sperm quality while there was a positive correlation between the male who undergoes the varicoceloctomy process and improvement of sperm parameters (Vivas-Acevedo *et al*., 2010).

The reduction of mentioned sperm parameters might respond to the increase of intravenous diameter in patients with Varicocele Grade3 compared to patients with Varicocele with Grade1 and Grade2 which caused increased level of active oxygen species and resulting increase sperm immature (Villanueva-Diaz *et al*., 1999). This resultsagree with previous studies, which found a direct correlation between ROS and severity of injury and the increasing of reactive oxygenic species decrease the quality of sperm parameters (Zini*et al*., 1998; Musalam*et al*., 2010).

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Table 2 showed a significant increase in ROS in infertile men seminal plasma complaining of varicocele grad 3, in comparison with grad 2 and grad 1 specimens. The oxidative stress is directly related to varicocele, and this result agree with Allamaneni*et al*. (2004), showed that semen reactive oxygen species levels correlated positively with the grade of the varicocele. Also in another research by Koksal*et al*.,(2007) revealed that oxidant stress is elevated in patients complaining of varicocele and that the level depended on the severity of varicocele, and the highest level seen in patients suffering of grade 3 varicocele and this finings agree with current study.

Also the results showed the presence of a significant increase in MDA concentration in patients varicocele Grade2 and Grade3 compared to patients varicocele Grade1 and perhaps due to increasing of the immature sperm and which are related with the production of reactive oxygen species ROS and may be related to increase in leucocytes in Grade2 and Grade3 that responsible for the production of ROS too (Koksal*et al*., 2003), and this results consistent with previous studies conducted infertile male with varicocele that found there is a positive relation between the grade of the varicocele and the level of MDA (Allamaneni*et al*., 2004; Mostafa *et al*., 2012), and negative correlation with sperm function test (Das *et al*. 2009; Hendin*et al*., 1999).

The results showed a significant reduction in SOD1, GSH and CAT concentration of infertile seminal plasma complaining of varicocele compared to fertile men seminal plasma and this result agree with a study showed significantly reduced levels of enzymatic antioxidants components in the seminal plasma of infertile men which suffering of varicocele (Abd-Elmoaty*et al*, 2010), this study also noted seminal OS levels were higher in the patients with high grades of varicocele.

The results show significant difference between the percentage of DNA integrity of patients with varicocele Grade1, Grade2 and Grade3 may be as a results of all mitochondria is active in Grade1 while all be inactive in Grade3 may respond the reason to the high level of ROS and this leads to an oxidative stress, and increase the damage chromatin or DNA of sperm (Lacerda*et al*., 2011). The results of the present study consistent with previous studies where found the percentage of the DNA integrity is high proportion in patients with varicocele Grade1 than it is in patients with varicocele Grade2 and Grade3 and may be the varicocele cause affect the tissues of the testis of in terms of impact the membrane and increased permeability (Lacerda*et al*., 2011; Vignera*et al*., 2012).

It was concluded the severity of varicocele can lead to a significant harmful effect on sperm parameters and the concentration of MDA, ROS, GSH, SOD1, CAT and chromatin maturity percent and level of inhibin b in serum and seminal plasma of infertile male which complaining of varicocele.

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