VITAMIN D_3 ADMINISTRATION BEFORE AND AFTER DETORSION COULD SALVAGE THE TESTICULAR ENDOCRINE FUNCTION IN AN EXPERIMENTAL MODEL OF TESTICULAR TORSION/DETORTION

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ABSTRACT:

Background: *Testicular torsion is one of the emergencies affecting mostly adolescent males, resulting in sub-fertility if not rapidly and efficiently managed. Oxidative stress, inflammatory response as well as immunological reactions were implicated in its pathogenesis. Recently interest in studying the non-skeletal effects of vitamin D is growing, many researches indicates its antioxidant, anti-inflammatory and immuno-modulatory effects.*

Aim of the Work: *Investigating the possible conservative effect of vitamin D_3 treatment on testicular endocrinal function in testicular torsion/detorsion rat model, and elucidating its possible underlying mechanism.*

Materials and Methods: *24 young adult male albino rats, weighing 120-160 g., were randomly allocated into 3 groups, 8 in each group, Sham operated (SHAM), Testicular torsion/detorsion (T/D) and Testicular torsion/detorsion; vitamin D_3 treated (T/D; D_3) groups. All rats were subjected to measurement of absolute and relative testicular weight (ATW and RTW, respectively), assessment of testicular endocrinal function by serum total testosterone and inhibin B levels. In addition to determination of serum antisperm antibody (AsAb). Testicular tissue was examined for oxidative stress markers; malondialdeyde (T. MDA) and glutathione peroxidase (T, GPx), as well as inflammatory marker; myeloperoxidase (T. MPO).*

Results: *Testicular T/D resulted in significant reduction in ATW, RTW, serum testosterone and inhibin B levels with significant elevation in serum AsAb, T. MDA and T. MPO when compared with SHAM group. On treatment with vitamin D_3, RTW was significantly increased as compared with T/D group but still significantly less than SHAM group. Serum testosterone level was significantly increased when compared with both SHAM and T/D groups. Inhibin B was significantly increased as compared with T/D group but still not normalized as being significantly less than SHAM group. Serum AsAb, T. MDA and T. MPO were significantly decreased when compared with T/D group, being normalized for T. MDA but still significantly higher for serum AsAb and T. MPO when compared with SHAM group, however, T. GPx was significantly increased as compared with both SHAM and T/D groups.*

Conclusion: *Vitamin D_3 could retrieve the testicular endocrinal dysfunction induced by Torsion/Detorsion by its antioxidant, anti-inflammatory and immuno-modulatory effects.*

Key words: *Testicular T/D, Vitamin D_3, Testosterone, Inhibin B, AsAb, oxidative stress and inflammation.*

INTRODUCTION:

Testicular torsion is a serious urologic emergency observed in adolescent males, with annual incidence of 1/4000 of the male population younger than 25 years old[(1)]. Delayed or misdiagnosis frequently result in loss of the ipsilateral testis, which may cause decreased spermatogenesis in 50% to 95% of patients, reducing fertility rates[(2)]. Further, testicular atrophy following torsion of the spermatic cord has been reported to increase the risk of testicular cancer[(3)].

The ischemia/reperfusion injury due to testicular Torsion/Detorsion (T/D) has been implicated in the pathogenesis of testicular damage[(4)]. Testicular torsion results in ischemia and necrosis of the testicular tissue[(5)]. Prompt surgical exploration and detorsion can contribute to salvaging the testis but permanent damage can occur, depending on the degree and duration of the torsion[(6)].

Reperfusion of the ipsilateral testis after ischemia results in overproduction of reactive oxygen species[(7)], cytokines including interleukin-1β and tumor necrosis factor-α, which cause recruitment of macrophages and neutrophils that infiltrate the testicular parenchyma[(8)], these could lead to apoptosis of germinal cells[(9)], and impair the function of Sertoli cells[(10)], leading to deterioration of spermatogenesis and testicular atrophy[(8,11)].

Some agents have been applied to reduce testicular T/D injury in experimental animal models, however, the effect of vitamin D_3 has not been evaluated yet. Vitamin D_3 (1, 25-Dihydroxy Cholicalciferol; DHC) is most commonly associated with the regulation of calcium homeostasis, the broad distribution of vitamin D receptor (VDR) suggest that vitamin D may have a much broader spectrum of activity, becoming an emerging therapeutic strategy for diseases beyond bone metabolism. Recent studies have demonstrated that vitamin D is an important factor playing a crucial role in inflammation, immunomodulation, detoxification, autophagy and apoptosis[(12,13,14)].

The presence of VDR in testicular tissues ensures that vitamin D has a role in the male reproductive functions[(15)]. Many human studies have also demonstrated an association between serum vitamin D concentrations and gonadal hormone production[(16,17)]. But some other studies have failed to document this correlation[(18,19)].

Therefore, this study aimed to investigate the potential effect of vitamin D_3 in alleviating the testicular endocrine derangement in an experimental model of Torsion/Detorsion injury in rats and to elucidate its possible underlying mechanism.

MATERIALS AND METHODS:

This study was carried on (24) male albino rats initially weighing 120-160 g. Animals were housed in clean animal cages, 5 animals per cage, with suitable ventilation, temperature of 22-25^{o}C, free access to food and water ad libitum, at Medical Ain Shams Research Institute (MASRI), Faculty of Medicine, Ain Shams University. The animals were allowed to the new environment for 7 days prior to experimental procedures to decrease the possible discomfort of animals. Animal manipulation was performed with maximal care and hygiene without exposure to unnecessary pain or stress. Surgical procedure was done under anesthesia to avoid induction of pain.

Experimental Groups:

Rats were randomly allocated into 3 groups, 8 rats in each: Sham operated group (SHAM); Rats subjected to all surgical steps as the other 2 groups except for torsion/detorsion, Testicular Torsion/ Detorsion group (T/D); Rats subjected to

720° torsion for 2 hours then detorsion for 30 days with sesame oil injection subcutaneously as a vehicle to vitamin D_3, Testicular torsion/detorsion; vitamin D_3 treated group (T/D; D_3); Rats subjected to 720° torsion for 2 hours then detorsion for 30 days with subcutaneous injection of vitamin D_3 in a dose of 500 IU/ Kg/day[20], starting half an hour before detorsion, then given daily, 5 days/week, for 30 days.

Drugs:

Vitamin D_3 was obtained as Devarol ampoule, 5mg/2ml (200.000 IU/2ml), supplied by CHEMIPHARM pharmaceuticals industries, Egypt. Sesame oil was supplied by El Hawag for Natural Oils Company, Egypt. 0.5ml of devarol ampoule (50.000 IU) was dissolved in 99.5ml sesame oil to reach final concentration of 500 IU/1ml, injected subcutaneously as 0.1ml/100g. rat B.W.

Experimental Procedures:

Testicular Torsion/Detorsion animal model: After being anaesthetized with ether inhalation, rats were fixed on the table on their back, then the skin of the scrotum was disinfected with betadine solution, and all procedures were performed under sterile conditions. A left vertical paramedian incision was made on the scrotum and the left testis was exposed, then was manually rotated 720˚ clockwise (two cycles of full rotation) to perform torsion, fixed by clipping as shown in figure (1), then covered by a piece of cotton soaked with normal saline. After 2 hours the left testis was rexposed, detorted and placed back in its anatomical position. The scrotal incision was closed with 2/0 silk suture[21].

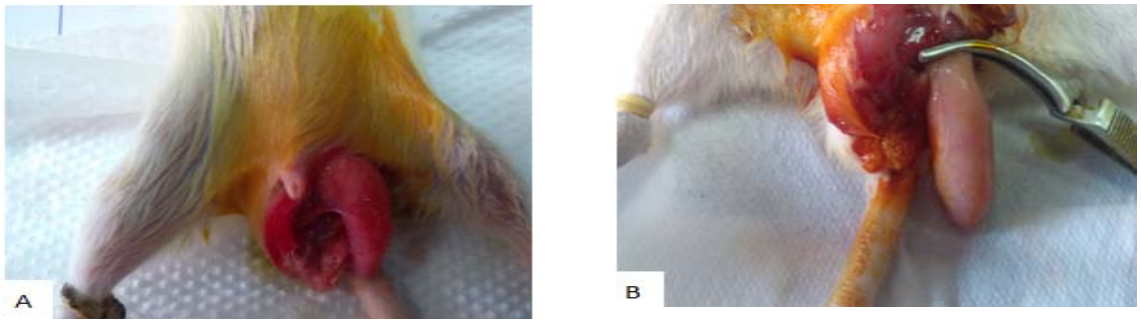


Figure (1): Left testicle exposure (A) then 720˚ clockwise rotation followed by clipping (B).

After 30 days, overnight fasted rats were anaesthetized with pentobarbital in a dose of 40 mg/ Kg B.W., retroorbital samples were collected in a plain tube and centrifuged at 3000 rpm for 15 minutes. Then, serum was separated and stored at -80°C for later determination of total testosterone, inhibin B and antisperm antibody (AsAb). The scrotum was reopened to extract the left (ipsilateral) testis, that was weighed then stored at -80°C for later biochemical analysis of testicular malondialdehyde (T. MDA), glutathione peroxidase (T. GPx) and myeloperoxidase (T. MPO).

Assessment of testicular endocrinal function by measuring serum level of total testosterone using Steroid EIA (enzyme immunoassay)-Testosterone, ALKPR-BIO, France. Inhibin B was measured by rat specific inhibin B ELISA (enzyme linked immunosorbant assay) kit, My Bio Source, USA.

Estimation of immunological reaction by assessment of serum AsAb using rat specific ELISA kit, Cube Biosystems, College Park, USA.

Determination of testicular oxidative stress markers colormetrically using MDA OxiSelect "TBARS; thiobarbituric acid reactive substances" assay kit, CELL BIOLABS, USA, and, GPx assay kit, Cayman Chemical, Ann-Arbor, USA.

Assessment of testicular inflammatory response by measuring MPO using rat specific CLIA (chemiluminescent immune-assay) kit, Life Span Bio Sciences, Seattle, USA.

Statistical Analysis:

All statistical data and statistical significance were performed using SPSS (statistical program for social science) statistical package (SPSS Inc.) version 20. Differences between groups were compared by one way ANOVA with least significant difference test (LSD) to find inter groupal significance. The association between the parameters was determined using the Pearson's correlation coefficient. $P \leq 0.05$ was considered statistically significant.

RESULTS:

As shown in table (1), both ATW and RTW were significantly decreased in T/D group when compared with SHAM rat group. In T/D; D_3 group, ATW and RTW were increased but the increase was only significant for RTW when compared with T/D group, but still significantly less than SHAM group.

Table (1): Mean ± SEM values of Final Body Weight (FBW; gm), Absolute Testicular Weight (ATW; gm) and Relative Testicular Weight (RTW; %) in all studied groups.

Parameters \ Groups	SHAM	T/D	T/D; D_3
FBW	169.00±5.28	188.70±3.94 [a]	174.30±7.19
ATW	0.86±0.05	0.58±0.03 [a]	0.72±0.08
RTW	0.51±0.02	0.30±0.01 [a]	0.41±0.04 [ab]

a: Significance by LSD from SHAM group with P≤0.05.
b: Significance by LSD from T/D group with P≤0.05.

Significant reduction in serum total testosterone and inhibin B levels, with significant elevation in serum level of AsAb were shown in T/D group as compared to SHAM group. However, T/D; D_3 group showed significant elevation in total testosterone when compared with both SHAM and T/D groups, with significantly increased inhibin B level and significantly decreased AsAb level when compared with T/D group but both not normalized when compared with SHAM group as shown in table (2).

Table (2): Mean ± SEM values of serum total Testosterone (Testosterone; pg/ml), Inhibin B (pg/ml) and Anti Sperm Antibody (AsAb; ng/ml) in all studied groups.

Parameters \ Groups	SHAM	T/D	T/D; D_3
Testosterone	123.10±6.41	62.70±2.75 [a]	161.90±4.09 [ab]
Inhibin B	38.60±1.31	22.30±1.01 [a]	28.85±1.38 [ab]
AsAb	0.72±0.03	1.80±0.12 [a]	0.95±0.03 [ab]

a: Significance by LSD from SHAM group with P≤0.05.
b: Significance by LSD from T/D group with P≤0.05.

Left (ipsilateral) testicular tissue was examined for MDA, GPx and MPO, all showed significant elevation in T/D group when compared with SHAM group except for T. GPx whose elevation was insignificant. On vitamin D_3 treatment T. MDA was significantly decreased when compared with T/D group being normalized when compared with SHAM group, T. GPx was significantly elevated when compared with both SHAM and T/D groups. T. MPO showed significant reduction as compared to T/D rat group, but still significantly higher than SHAM group.

Table (3): Mean ± SEM values of testicular tissue malondialdehyde (T. MDA; umol/gm), glutathione peroxidase (T. GPx; mg/gm) and myeloperoxidase (T. MPO; pg/gm) in all studied groups.

Parameters \ Groups	SHAM	T/D	T/D; D_3
T. MDA	284.64±14.33	4946.13±671.08 [a]	695.56±83.62 [b]
T. GPx	0.27±0.01	0.59±0.06	3.13±0.32 [ab]
T. MPO	36.20±2.03	92.50±2.78 [a]	75.45±1.44 [ab]

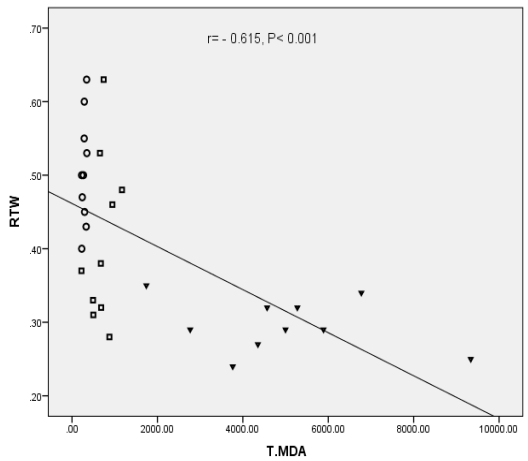
a: Significance by LSD from SHAM group with P≤0.05.
b: Significance by LSD from T/D group with P≤0.05.

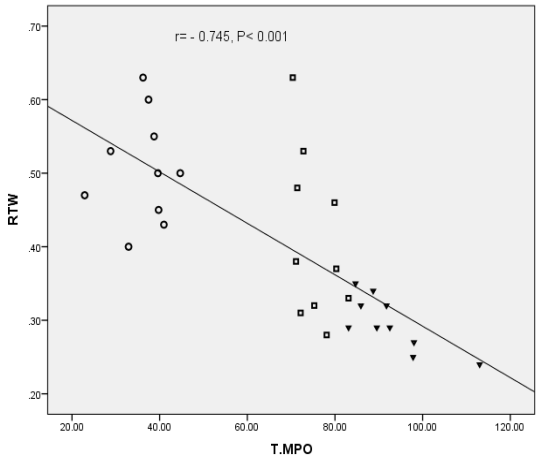
Correlation study:

A correlation study revealed a negative correlation between each of T. MDA, T. MPO, S. Anti-Sperm Ab and RTW (r= - 0.615, - 0.745, - 0.633 respectively), all at P<0.001. Also, a significant positive correlation between RTW and each of S. testosterone level (r= 0.432, P<0.05), S. inhibin B level (r= 0.746, P<0.001) was detected, as shown in figure (2).

Meanwhile, each of T. MDA, T. MPO and S. Anti-Sperm Ab showed significant negative correlation with S. testosterone level (r= - 0.755, P<0.001), (r= - 0.393, P<0.05) and (r= - 0.702, P<0.001) respectively. However, T. GPx showed a significant positive correlation with S. testosterone (r= 0.660, P<0.001), as shown in figure (3).

Each of T. MDA, T. MPO and S. Anti-Sperm Ab showed a significant negative correlation with S. inhibin B (r= - 0.640, - 0.874, - 0.729 respectively), all at P<0.001). Morover, S. inhibin B showed a positive correlation with S. testosterone level (r= 0.403, P< 0.05), as shown in figure (4).





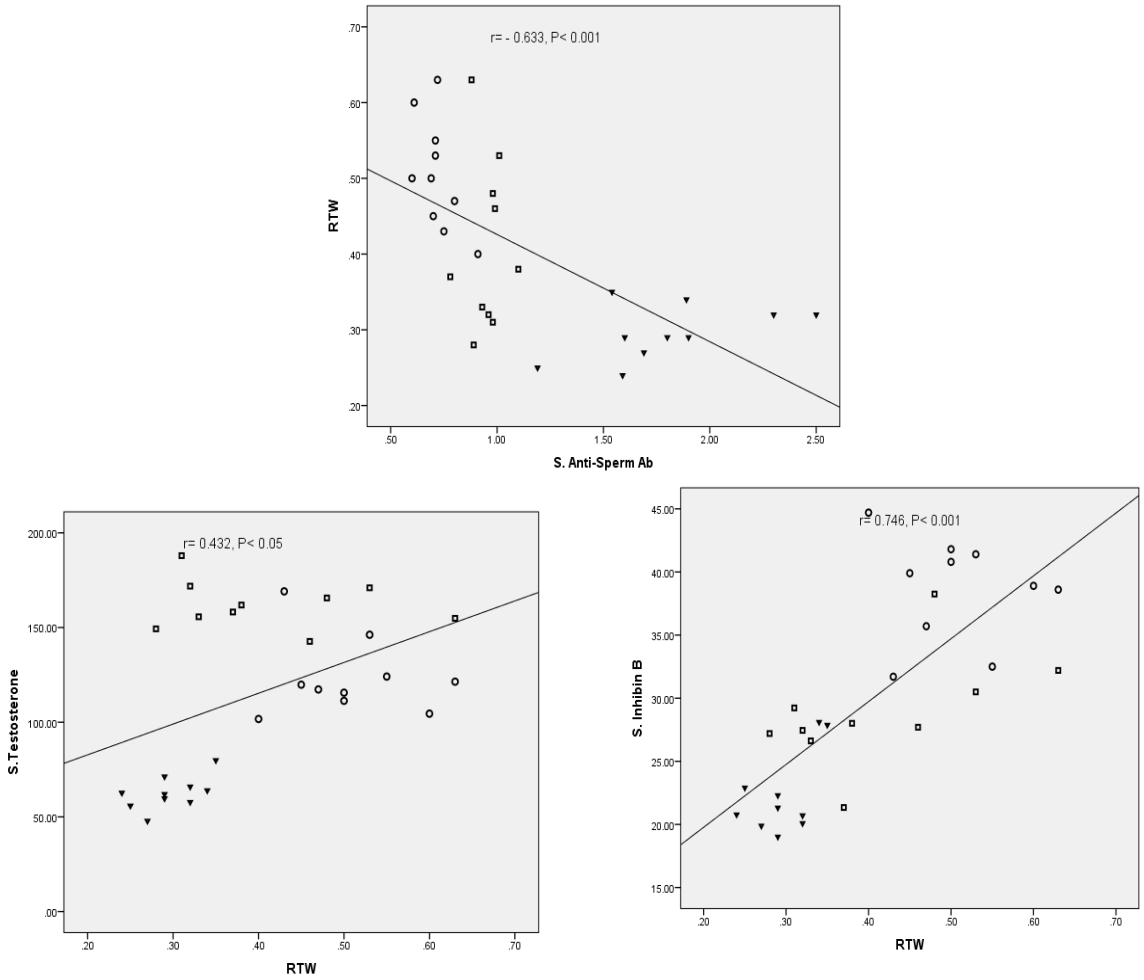
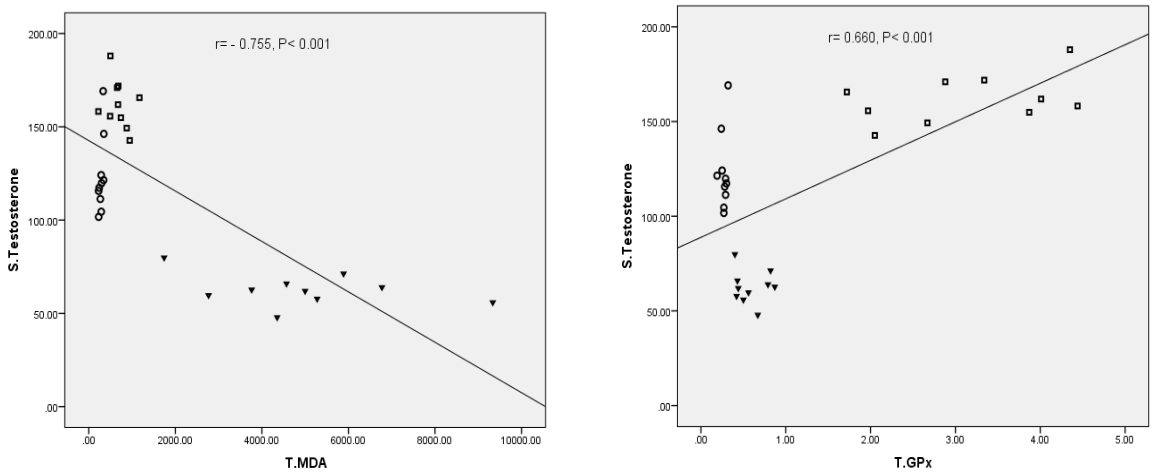


Figure (2**):** Correlations between relative testicular weight (RTW) and each of testicular malondialdehyde (T. MDA), myeloperoxidase (T. MPO), serum antisperm antibody, testosterone and inhibin B in all studied groups.
○: SHAM group, ▼: IR group, □: IR; D_3 group



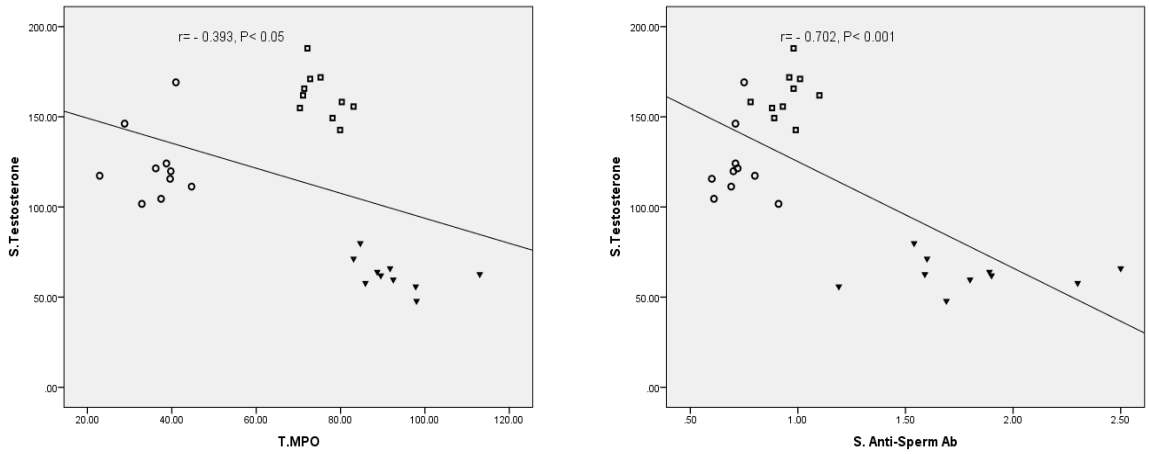


Figure (3): Correlations between each of testicular malondialdyhyde (T. MDA), glutathione peroxidase (T. GPx), myeloperoxidase (T. MPO), serum antisperm antibody and testosterone in all studied groups.

○: SHAM group, ▼: IR group, □: IR; D_3 group

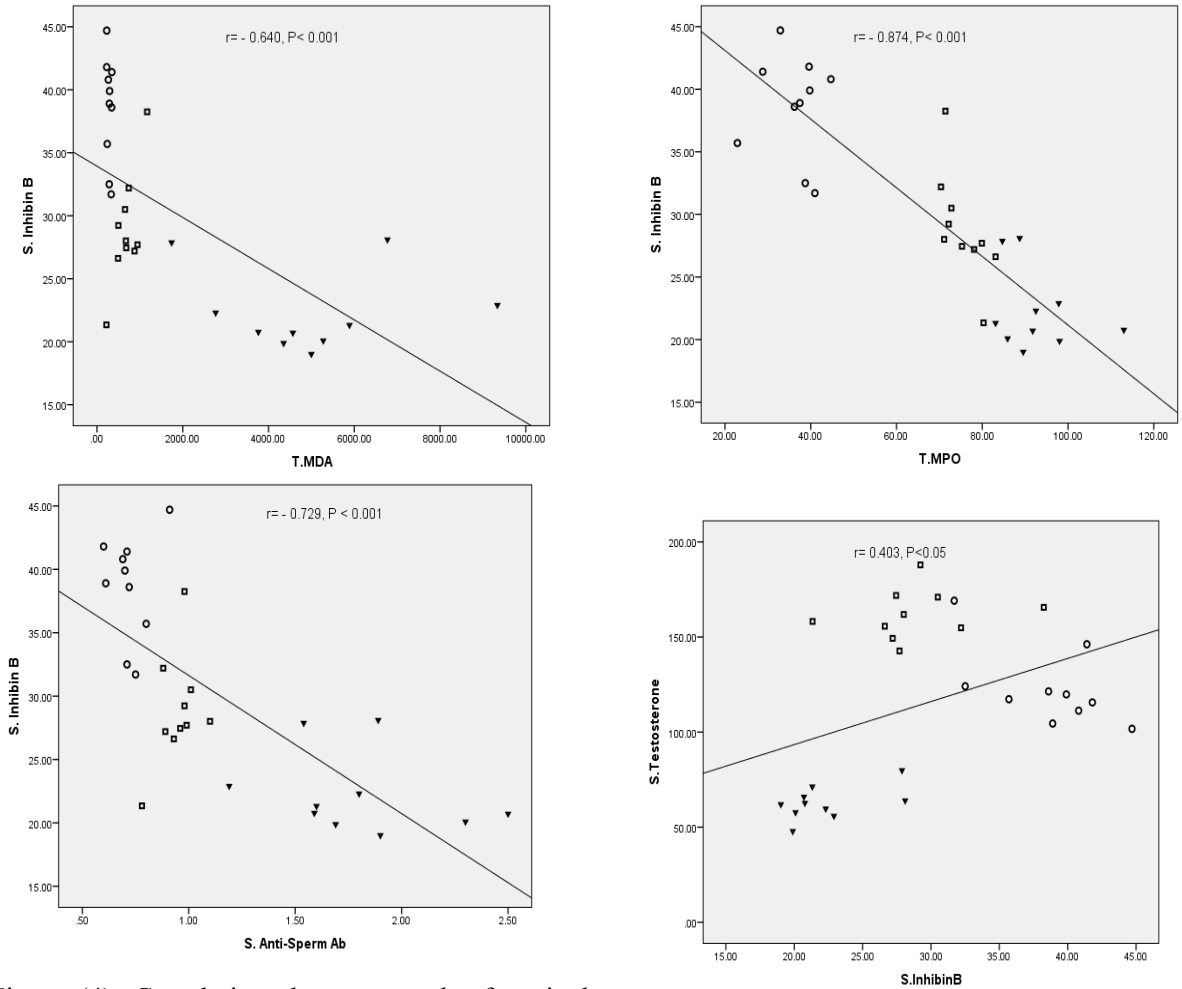


Figure (4): Correlations between each of testicular malondialdyhyde (T. MDA), myeloperoxidase (T. MPO), serum antisperm antibody, testosterone and inhibin B in all studied groups.

○: SHAM group,▼: IR group, □: IR; D_3 group

DISCUSSION:

Testicular Torsion/Detorsion (T/D) is the commonest urological emergency in young adolescent males, in which oxidative stress, inflammation and immunological responses were implicated in its pathogenesis. In this work vitamin D_3 was chosen because of its recently discovered extra-skeletal effects, aiming to assess the effectiveness of its antioxidant, anti-inflammatory and immunological activities in testicular T/D model. Various parameters such as testicular weight, serum testosterone, inhibin-B and antisperm antibody (AsAb), as well as, testicular tissue malondialdehyde (T. MDA), glutathione peroxidase (T. GPx) and myeloperoxidase (T. MPO) were assessed.

This study was carried on 3 months aged male rats representing the target age group, during which the risk of torsion increases as testicular volume increases at a faster rate than the mesenteric structure during puberty as stated by Hyun[22]. Previous studies have shown that 30 min to 1 hour of torsion, and then 1 to 4 hours of detorsion is enough to successfully form a testicular T/D model[23,24]. Therefore, we established a model in which a 2 hour testicular torsion of 720° degrees clockwise rotation of the left testis was followed by a 30 days detorsion.

The reduced absolute and relative testicular weight (ATW and RTW respectively) in T/D testicles either treated or not could be explained by the reduced blood supply during the torsion period, as well as the oxidative stress and leukocytic infiltration following its detortion as indicated by high testicular MDA and MPO levels, in addition to the autoimmune reaction against the testicular tissue as indicated by the elevated serum AsAb, this is confirmed by the significant negative correlation between each of T. MDA, T. MPO, serum AsAb and RTW. This is in accordance with Wei et al.[25] who stated that testicular T/D produced pronounced injury in the ipsilateral testis 3 months after detorsion, including significant decreases in testicular weight, mean seminiferous tubular diameter, germ cell layer number and mean testicular biopsy score. Previously, Mansbach et al.[26] reported that complete or severe testicular atrophy was detected in all patients with cord twisting higher than 360°.

The reduced serum testosterone and inhibin B level observed in T/D rat group is indicative to testicular tissue damage, this is in accordance with Romeoa et al.[27] who demonstrated that testicular torsion is responsible for late impairment of both exocrine and endocrine testicular functions.

Inhibin B has been proposed as a direct marker of Sertoli cell function and indirect marker of spermatogenesis[27,28], and can be correlated with testis volume and testosterone production[29], this was proved in this study by the significant positive correlation between serum inhibin B and testosterone levels. Reduction of inhibin B has been ascribed to an early arrest of spermatogenesis or a depletion of germ cells and associated with a reduced testicular volume[29]. This was confirmed in this study by the significant positive corelation between RTW and both S. testosterone and inhibin B.

This testicular dysfunction could be due to oxidative stress, inflammation and autoimmune response as indicated by the significant elevation in T. MDA, T. MPO, serum AsAb with insignificantly elevated T. GPx in T/D rat group, as well as the significant negative correlation between each of T. MDA, T. MPO, serum AsAb and each of serum testosterone and inhibin B.

Aitken and Roman[30] stated that both spermatogenesis and Leydig cell steroidogenesis are vulnerable to oxidative stress. Significantly increased T. MPO in T/D could be explained by damage to

vascular endothelium after ischemia/ reperfusion of tissue leads to upregulation of E-selectin expression on endothelial cells[31], with subsequent neutrophil adhesion to vascular endothelium and transmigration through vascular endothelium into tissue and release reactive oxygen species that cause tissue injury[32]. MPO is found predominantly in the granules of neutrophils. Therefore, the enzyme activity is used as an indicator of neutrophil accumulation in tissue[33]. Moreover, the increase in proinflammatory cytokines (TNF-α, IL-1β, and IL-8) after ischemia/ reperfusion results in recruitment of neutrophils and macrophages and infiltration of the testis by inflammatory cells[8]. These changes affect the function of Sertoli cells, cause apoptosis of germinal cells[10], impaired spermato-genesis and result in testicular atrophy[11].

Elevated serum level of AsAb could be explained by the immunological reaction secondary to blood testicular barrier damage following testicular T/D. The blood–testis barrier physiologically isolates most immune cells out of the testicular tissue and limits the inflammatory response[34]. Autoimmune response, triggered by blood test is barrier breakdown, secondary to ischemic damage leading to exposition of antigenic material and formation of antibodies against testicular elements[35].

Vitamin D_3 administration prior to and long after detorsion significantly minimized the functioning cell loss as evidenced by not only restoration of serum testosterone level but also its significant increase even from sham group despite the still significantly decreased RTW, this might indicate the enhanced stimulatory effect of vitamin D_3 on leydig cells possibly by elevation of FSH, LH level (we didn't follow testosterone hormone changes or assess the hypothalamic-pituitary-gonadal axis in this study), or a possible stimulant effect of vitamin D_3 on Leydig cells. The partial improvement in inhibin B level may reflect the less number of vitamin D receptors on Sertoli cells (i.e. less sensitivity to vitamin D_3). Vitamin D_3 restored the oxidant/ antioxidant balance toward normal, improving the inflammatory outcome and reducing the AsAb level that indicates either immune reaction alleviation or restoration of testicular blood barrier integrity with lack of continuous sensitization of the immune system to testicular proteins which would be beneficial in the context of limiting the immune attack of testicular tissue. The outcome of targeting all these mechanisms minimized the risk of testicular damage and dysfunction.

The observed marked elevation of serum testosterone level on vitamin D_3 treatment is in accordance with Wehr et al.[36], the researchers found that men with sufficient vitamin D levels had significantly higher levels of testosterone and free androgen index and significantly lower levels of sex hormone binding globulin when compared to vitamin D deficiency. Moreover, Hajhashemi et al.[37] demonst-rated a positive association between plasma level of 25OH vitamin D and testosterone level. The elevated inhibin level is in accordance with Blomberg Jensen et al.[38] who demonstrated a positive association between vitamin D status and inhibin B levels in infertile men. Moreover, Bouillon et al.[39] demonstrated that VDR-knockout mice presented with hypogonadism, low sperm count and motility, and microscopic abnormalities of testicular tissues.

Hofer et al.[40] reported that the primary human testicular cells which were treated with vitamin D_3 showed a marked increase in testosterone production and enhancement in the expression of mRNA of enzymes involved in testosterone production and its precursors. Moreover, Hussain et al.[41] returned the testosterone-stimulatory effect of vitamin D_3 to the maintenance of the levels of Luteinizing Hormone (LH), and Follicle Stimulating Hormone (FSH), as well

as, up-regulating superoxide dismutase, glutathione peroxidase, and the antioxidant pool, with down-regulating malondial-dehyde, inducible nitric oxide synthase species and nitric oxide.

Antioxidant effect of vitamin D is between the newest suggested non-calcemic roles of this compound. In a study conducted on rats, it was found out that the antioxidant effects of vitamin D were similar or even further than vitamin E[42]. In the present study, vitamin D treatment decreased the T. MDA level but still higher than SHAM group, and significantly increased GPx, an important antioxidant enzyme that rapidly converts hydrogen peroxide (H_2O_2) to water and preventing its accumulation.

This is in accordance with Mokhtari et al.[43] who stated that vitamin D induces the expression of several molecules involved in the antioxidant defense system including reduced glutathione (GSH), glutathione peroxidase and superoxide dismutase and suppresses the expression of NADPH oxidase. Moreover, vitamin D_3 could enhance the pathway of reactive oxygen species removal, by increasing the intracellular pool of reduced GSH, partially through upstream regulation of glutamate-cysteine ligase (GCL) and glutathione reductase genes expression[44]. GCL is a key enzyme that involved in synthesis of GSH[45]. A positive correlation between the vitamin D and GSH concentrations have been reported by Jain et al.[46].

The significant reduction in T. MPO and S. AsAb observed in the treated group indicates the anti-inflammatory and immuno-modulatory effect of vitamin D. Helming et al.[47] reported that vitamin D induces monocytic differentiation to macrophages and reduces the release of inflammatory cytokines and chemokines by these cells. In addition, Griffin et al.[48] stated that 1, 25 $(OH)_2D_3$ has stimulatory effect on phagocytosis and suppresses its antigen-presenting capacity. Also, 1, 25 $(OH)_2D_3$ suppresses autoantibody production as stated by Linker-Israeli et al.[49]. Therefore, vitamin D has an important role in increasing the effects of innate immune processes while restraining the adaptive immune system, leading to improved outcomes in autoimmune diseases[50,51].

Based on the results of the present study, it is highly recommended to administer vitamin D_3 prior to and long after detorsion to salvage the endocrine testicular function. Further studies for hormone changes follow up of the hypothalamic-pituitary-gonadal axis, as well as trial of different doses and durations of vitamin D_3 are needed.

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إعطاء فيتامين (د) قبل وبعد الإسترداد ينقذ وظيفة الغدد الصماء للخصية في نموذج تجريبي لإلتواء/إسترداد التواء الخصية

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الخلفية: التواء الخصية هي واحدة من الحالات الطارئة التي تحدث في الذكور بسن المراهقه ، والذى يؤدى إلى نقص الخصوبة إذا لم تتم معالجتها بسرعة وكفاءة. ويتورط كل من الإجهاد التأكسدي ، الإستجابة الإلتهابية وكذلك ردود الفعل المناعية في التسبب في المرض. وتزايد الإهتمام بدراسة الآثار غير الهيكلية لفيتامين (د) في الآونة الأخيرة ، تشير العديد من الأبحاث إلى آثاره المضادة للأكسدة والمضادة للالتهابات والإستجابه المناعية.

الهدف من العمل: يهدف هذا العمل إلى توضيح التأثير الوقائى المحتمل لإعطاء فيتامين (د) على نموذج تجريبي لإلتواء/إسترداد التواء الخصية فى الجرذان.

المواد والطرق: تم تخصيص ٢٤ من ذكور الجرذان البالغين الصغار، وزنها ١٢٠-١٦٠ جم، وقسمت بشكل عشوائي إلى ٣ مجموعات، ٨ في كل مجموعة ، المجموعه الضابطه ، مجموعه التواء/إسترداد التواء الخصية ومجموعه التواء/إسترداد التواء الخصية المعالجه بفيتامين (د). وتعرضت جميع الفئران لقياس وزن الخصية المطلق والنسبي، وتقييم وظيفة الخصية كغده صماء بواسطة قياس مستوى هرمون التستوستيرون الكلي و إنهيبين ب في الدم. بالإضافة إلى تحديد الأجسام المضادة للحيوانات المنويه بالدم. كما تم فحص أنسجة الخصية لكل من علامات الإجهاد التأكسدي المالونديالديهايد والجلوتاثايون براوكسيديز، وكذلك علامة الالتهابات المايلوبراوكسيديز.

النتائج: أدى التواء/إسترداد التواء الخصية إلى انخفاض ذو دلاله إحصائيه في الوزن المطلق والنسبي للخصية ، مستويات هرمون التستوستيرون و إنهيبين ب في الدم وارتفاع ذو دلاله إحصائيه في الأجسام المضادة للحيوانات المنويه بالدم، وزيادة في أنسجة الخصية لكل من المالونديالديهايد و المايلوبراوكسيديزعند مقارنتها مع المجموعة الضابطه. و مع العلاج بفيتامين (د) لوحظ زيادة فى الوزن النسبى للخصيه بشكل ذو دلاله إحصائيه مقارنةً بمجموعة التواء/إسترداد التواء الخصية ولكن لا يزال أقل من المجموعة الضابطه. كما زاد مستوى هرمون التستوستيرون في الدم بشكل ملحوظ عند مقارنته بكل من المجموعه الضابطه ومجموعه التواء/إسترداد التواء الخصية. مع زيادة فى مستوى الإنهيبين ب بالدم بشكل ملحوظ مقارنةً بمجموعة التواء/إسترداد التواء الخصية ولكن لا يزال أقل بكثير من المجموعة الضابطه. وكذلك إنخفض مستوى الأجسام المضادة للحيوانات المنويه بالدم وكلا من المالونديالديهايد والمايلوبراوكسيديز بنسيج الخصية بشكل ذو دلاله إحصائيه عند مقارنتهما بمجموعة التواء/إسترداد التواء الخصية، حيث رجع للمستوى الطبيعى بالنسبه للمالونديالديهايد ولكن لا يزال مستوى الأجسام المضادة للحيوانات المنويه بالدم والمايلوبراوكسيديز بنسيج الخصيه أعلى بشكل ملحوظ عند مقارنتهما بالمجموعة الضابطه ، كما زاد مستوى الجلوتاثايون براوكسيديززيادة كبيرة من خلال علاج فيتامين (د) مقارنة مع كل من المجموعه الضابطه ومجموعة التواء/إسترداد التواء الخصية.

الخلاصة: يمكن لفيتامين (د) أن يخفف من إختلال وظيفة الغدد الصماء للخصية الناجم عن التواء/إسترداد التواء الخصية بسبب آثاره المضادة للأكسدة والمضادة للإلتهابات والمعدلة للإستجابه المناعية.