POTENTIAL ROLE OF CADMIUM IN VARICOCELE-ASSOCIATED INFERTILITY AND ITS RELATION TO SMOKING

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ABSTRACT

Objectives: *to study the possible role of cadmium in varicocele-associated infertility and its relation to smoking.*
Patients: *the study was performed on twenty infertile men with clinically evident varicocele (grade II & III); half of them were smokers. In addition to another twenty fertile men without clinical varicocele and with normal semen parameters served as a control; half of them were smokers*
Methods: *measuring Cadmium level in the seminal plasma using the atomic absorption spectrophotometer.*
Results: *Statistically significant observed differences in seminal parameters between the studied cases and their control regarding decreased sperm concentration and motility, in addition to increased abnormal sperm morphology among cases (P=0.0001, 0.0001 and 0.02 respectively). A statistically significant high level of cadmium is demonstrated in seminal plasma of non smoking cases as compared with their non smoking control (p<0.0001), and in seminal plasma of smoking control in comparison to non smoking control (p<0.002). Moreover, the level of cadmium detected in seminal plasma of infertile smokers was higher than that of smoking control (P=0.001). Partial correlation between cadmium and seminal parameters of the studied cases and control, in the present study, revealed that cadmium is correlated with sperm concentration and motility even after adjustment for smoking, non smoker/ even smoker, (P=0.009 and 0.003 respectively).*
Conclusions: *the current study supports that environmental exposure to cadmium leads to its accumulation in seminal plasma of patients with varicocele-associated infertility. High level of cadmium is associated with impairment of seminal parameters. In addition, cigarette smoking exacerbates the detrimental effect of varicocele on semen quality and the fertility potential by more accumulation of cadmium in seminal plasma.*
Key Words: *cadmium; varicocele, infertility; smoking.*

INTRODUCTION

Infertility is a reproductive health problem; it is defined as the inability to conceive after one year of unprotected intercourse,(1) It affects approximately 10-15% of reproductive-age couples. In 25% of cases, infertility is attributable to poor semen quality.(2) The etiology of poor semen quality is complicated. Increasing evidences suggest that chemical and physical agents in the environment may affect male fertility in humans.(3)

Heavy metals induced infertility

Mean sperm concentration and volume in normal males has dropped substantially over the past 50 years.(4) This decline in semen quality is believed to be related to environmental toxins and heavy metals exposure. Rapid industrialization, motorized vehicular traffic and population growth are believed to increase the toxic and heavy metals release into the environment.(5) Heavy metals exposure is associated with impaired semen quality due to their direct effect on testicular function as well as hormonal alterations. General population is exposed to cadmium which is present in contaminants found in drinking water, food and aerosol sprays that are present in cigarette smoke.(6) Cadmium is a non-essential toxic element, the exposure to it can adversely affect both male and female reproductive systems. Cadmium in seminal plasma may be increased by cigarette smoking as well as local nutritional or industrial exposure. Cadmium has a toxic effect on many enzymes that are dependent on iron as a co-factor, one of these being cytochrome P450. Leydig cells contain ten times more of cytochrome P450 than Sertoli cells. Thus Leyding cells are more sensitive to increased cadmium level.(7)

Cadmium-induced testicular damage is likely to occur as a three-phase process

Cadmium leads to disruption of blood-testis barrier (BTB) and germ cell loss, mediated through the activation of specific signal transduction pathways.(6) Cadmium induced effects occurs in three phases, five steps, as follows:

(i) Phase I:

Cadmium ions likely enter Sertoli and/ or germ cell via different mechanisms, such as diffusion, Ca2+ channels or using a Cd2+/Zn2+ transporter (step 1). Once inside the Sertoli and/ or germ cell, cadmium induces the synthesis and release of cytokines (e.g. TGFβ-3), which, by interacting with their respective receptors, activate the stress

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activated p38 MAPK, mitogen-activated protein kinase, signaling pathway (step 2) that can selectively disrupt the BTB.[6] At the same time, possibly also mediated by the p38 MAPK, the production of proteases (e.g. cathepsin L) is also induced (step 3). [6]

(ii) Phase II:

The BTB is disrupted via degradation of the integral membrane proteins (via the effects of proteases) and/ or an acceleration of clathrin-mediated endocytosis of tight junction (e.g. occludin) and basal proteins (e.g. N-cadherin). The net results of all this contribute to the disruption of the BTB and Sertoli–germ cell junctions that lead to germ cell loss (step 4).[6]

(iii) Phase III:

In order to limit unwanted proteolysis and avoid the dissolution of the entire seminiferous epithelium in the testis, there is an activation of c-JNK, jun-activated protein kinase c, signaling pathway, which, in turn, stimulates the production of protease inhibitors (e.g. α2-macroglobulin) to initiate recovery of the seminiferous epithelium (step 5).[6]

The most common gross morphological changes in the testis induced by cadmium are: hemorrhage and testis weight loss.[6] Cadmium induced histological injury on testis is disruption of the tight junctions in the microvessels which led to leakage of blood cells, most notably erythrocytes, into the interstitial space, causing hemorrhage and edema,[6] Furthermore, cadmium affected Leydig cells, thereby promoting a reduction in steroidogenesis, and cadmium also induced cell necrosis, for example, by production of reactive oxygen species. Taken together, these effects induced by cadmium exposure resulted in germ cell loss, causing irreversible sterility.[6]

Moreover, Cadmium has a role in production of varicocele-associated infertility through disrupting cytoplasmic actin organization. It was suggested that rising intratesticular cadmium level could be associated with both loss of actin filaments in cells of the seminiferous epithelium and increased level of germ cell apoptosis. Cadmium accumulation may disrupt the actin cytoskeleton, which in fertile men contributes to shaping of the acrosome around the sperm nucleus, second function of the sperm actin cytoskeleton is to effect acrosome exocytosis.[8] Cadmium in sperm appears to act as an effector that mechanically accounts simultaneously for oligospermia that accompanies varicocele, the stress sperm morphology seen in varicocele and the acrosome reaction insufficiency typical among men with varicocele.[8] The International Agency for Research on Cancer (IARC) has classified cadmium as a Category-I carcinogen.[9]

Smoking and male infertility

It was suggested that men with marginal semen quality may be pushed into the infertile range by smoking. Approximately one third of the world's population 15 years or older are active smokers, they smoke cigarettes daily. Needless to say, a number of non smokers are also negatively affected when they inhale side-stream smoke from burning cigarettes.[10] A causal relationship between cigarette smoking and impaired reproductive function is highly suspected because smokers inhale a host of toxins such as nicotine, carbon monoxide, cadmium, and other mutagenic compounds. The fact that nicotine and its water-soluble metabolite (cotinine) are detectable in the seminal plasma of smokers suggests that other harmful components of tobacco smoke would pass through the blood-testis barrier.[11]

Cigarette smoking is significantly correlated with increased levels of seminal oxygen species (OS); these oxidants deplete tissues and seminal fluid of antioxidants. The significant reduction of reactive oxygen species-total antioxidant capacity, ROS-TAC scores associated with smoking can be attributed to the significant increase in seminal ROS levels. The link between cigarette smoking and increased levels of seminal ROS may be, at least in part, related to the significant increase (48%) in leukocytes concentration in the semen of infertile smokers.[12] An additional factor that may explain why the semen of the smokers has increased levels of ROS may be the fact that cigarette smoke itself contains high levels of ROS such as superoxide anion, hydrogen peroxide and hydroxyl radicals.[12] Spermatozoa are particularly susceptible to damage induced by excessive ROS because their plasma membranes contain large quantities of polyunsaturated fatty acids and their cytoplasm contains low concentrations of scavenging enzymes.[13]

Varicocele and infertility

The most common identifiable cause of male subfertility is varicocele, a condition of palpably distended veins of the pampiniform plexus of the spermatic cord. It is caused mainly by retrograde blood flow through the internal spermatic vein. [1]

Epidemiology: Varicocele occurs in approximately 15% to 20% of the general male population, especially in adolescents. Varicocele occurs in 19% to 41% of men seeking infertility treatment and in around 80% of men with secondary infertility. Subclinical varicoceles are much more common than clinical varicoceles, being present in 44% of fertile men and up to 60% of men attending infertility clinics.[14]

Pathophysiology of varicocele-induced male infertility: The exact mechanism of impaired testicular function in patients with varicocele is not known yet. A number of theories have been proposed to explain the observed pathophysiology of varicocele including; hyperthermia, Reflux of renal

and adrenal metabolites, Hormonal dysfunction, Hypoxia, Abnormal blood flow, Antisperm antibodies, Genetic defect.[15]

Reactive oxygen species (ROS): There is increasing evidence pointing towards the role of ROS and oxidative stress in the pathogenesis of varicocele related subfertility. ROS production is usually high in those with a varicocele and improves after varicocelectomy. This increased ROS production can deplete the defenses present in the testes and in seminal plasma.[16]

Role of apoptosis in varicocele-induced male infertility:

Apoptosis, commonly referred to as programmed cell death, is the mechanism by which all cells die under normal conditions. Apoptotic cells are microscopically characterized by cell shrinkage, membrane blebbing, nuclear condensation, and fragmentation in the form of vesicles called 'apoptotic bodies'. Apoptosis is a normal physiological phenomenon in most tissues, and has a critical regulatory role. It is a prerequisite for normal spermatogenesis in mammals, and is thought to ensure cellular homeostasis, and to facilitate the maintenance of the balance between germ cells and Sertoli cells. Therefore, alterations in the apoptosis of germ cells may be crucial in varicocele-related human infertility.[17] Germ cell apoptosis during normal spermatogenesis is estimated to result in the loss of 25- 75% of potential mature sperm cells in the adult testes. Apoptosis appears to affect all three classes of germ cells, i.e. the spermatogonia, spermatocytes, and spermatids.[17]

Many pathways result in apoptosis, and these processes appear to be regulated on three levels:[18]

(i) In the cell membrane, specific membrane receptors mediate the death signals of the TNF receptor family, which consists of Fas and the Fas-ligand.

(ii) At the cytoplasmic level, signal transduction pathways involving cysteine proteases called caspases are also involved.

(iii) Finally, at the nuclear level, specific apoptotic regulatory genes, including p53 and Bcl-2, also exert regulatory effects on apoptosis.

Up-regulated expression of Fas protein, which is responsible for Fas-dependant apoptosis, in the semen of patients with varicocele is correlated, whereas little or no changes in Fas ligand expression were detected.[17] Furthermore, Up to 10% of sperm cells in the ejaculate of men with a varicocele were apoptotic, as compared with 0.1% in fertile men with no varicocele. That report concluded that varicocele induces apoptosis, which is initiated in the testicular tissue and is then expressed in the semen.[17] Morphological alterations in testicular tissues have been reported as 'stress patterns' in patients with varicoceles. This stress pattern is reminiscent of, although not identical to, the cytomorphological changes in apoptosis.[17] As well as there was far more apoptotic nuclei in the seminiferous tubules of men with varicocele. [19]

At least three pathways leading to apoptosis have been identified in animal models with varicocele:

(i) excess heat.[20]

(ii) androgen deprivation at the testicular level.[21, 22]

(iii) accumulation of toxic agents including the products of cigarette smoke such as cadmium.[23]

Role of cadmium in varicocele-induced apoptosis

Over time, testicular cadmium levels can accumulate. Increased cadmium levels on the testis side of the blood-testis barrier may exacerbate the effects of increased pressure, because cadmium itself alters the permeability of the barrier. This results in edema, rendering the blood-testis barrier more porous, and causing the testes to accumulate cadmium more rapidly.[8] It was suggested that cadmium results in markedly high level of apoptosis, ultimately culminating in oligospermia, based on detected high testicular cadmium level in patients with varicocele and its significant correlation with high number apoptotic cells within the seminiferous tubules. These values were concordant in both testes, despite the presence of a varicocele only on the left side. The values were inversely related to increases in sperm concentration occurring after varicocelectomy.[8]

Diagnosis of varicocele:

a. Clinical diagnosis: A careful medical and reproductive history is essential, and at least two semen analyses are required. However, varicocele is generally diagnosed on physical examination.[24, 25]

Clinical grading of varicocele:

Grade 0 (subclinical varicocele): Non-palpable reflux in the internal spermatic vein detected by Venography, Ultrasonography, or other non-invasive methodology.[26]

Grade I: Palpable distension detected only during a valsalva manoeuvre.[27]

Grade II: Palpable distension of the spermatic cord structures on the upright examination.[27]

Grade III: Visible distension of the spermatic cord structures on upright examination.[27]

b. Ultrasonographic diagnosis: Ultrasonography (US) is a widely used for structural and functional analysis of testicular tissue and can suggest a specific diagnosis for a wide variety of testicular diseases, and guiding treatment. Structural analysis is applied for measurement of testicular volume, studying of echo texture, and the illustration of tissue stiffness or elasticity. Functional analysis contains the illustration of macro and microvascularity, which are indicators of tissue perfusion.[28]

US-techniques:

1- Grey scale US: The diagnostic criteria of varicocele by gray scale US is ≥2 prominent veins in pampiniform plexus expand in a supine position with Valsalva maneuver and in upright position, one of which should have a diameter greater than 2-3 mm.[29]

2- Unenhanced Color Doppler US: Unenhanced CDUS is well established for determination of macro and microvascularity. These are good indicators of intratesticular perfusion, which seems to be related to testicular function. CDUS should be optimized to be sensitive to low velocity flow. CDUS can be used to grade venous reflux as static (grade I), intermittent (grade II), or continuous (grade III). The continuous reflux, lasting more than 2 seconds, is significant for diagnosis of subclinical varicocele. Intermittent and static refluxes, which are physiological, are insignificant if there is no palpable varicocele.[29]

Perfusion mapping, performed with the use of CDUS, has shown for the first time that in patients suffering from azoospermia, sperm quality and quantity depend on tissue perfusion within the testicle.[28] Power Doppler US uses the integrated power of the Doppler signal to depict the presence of blood flow. Higher power gains are more likely with power Doppler US than with standard color Doppler US, resulting in an increased sensitivity for detection of blood flow.[28]

3- Real-time sonoelastography (RTE): It was found that the elasticity pattern of the testis seems to be related to the volume and function. RTE can be used for targeted tissue sampling, like testicular sperm extractions (TESE).[28]

4- Advanced contrast agent detection techniques: In the last years different contrast agent detection techniques like the so-called CPS (cadence pulse sequencing technique) were proposed. All of these techniques are based on the principles of low MI (mechanical index) techniques to save the bubbles for a long time and to generate subtraction images to illustrate only the flow of the contrast media in the microvessels.[28]

Venography: Although venography is considered to be the gold standard, for diagnosis of varicocele, it is time consuming and invasive.[29]

Scrotal Thermography: Contact thermography using a thermovision infrared camera has a sensitivity of 84- 98% and a specificity of 81- 100 %.[30]

Others e.g. Radionuclide Angiography: The demonstration of varicocele by radionuclide angiography is accomplished by labeling the patient's erythrocytes with Technitium (Tc 99).[31]

Testicular biopsy: Varicoceles are associated with macroscopic and microscopic testicular damage, in addition to decreased testicular size. The testicular biopsy findings in varicoceles are classified histopathologically as normal spermatogenesis, hypospermatogenesis (mild, moderate, severe), maturation arrest (early, late), germinal cell aplasia with focal spermatogenesis, and total germinal cell aplasia, premature sloughing of germ cells into the seminiferous tubule lumen and Leydig cell hyperplasia.[32]A thickened basement membrane of seminiferous tubules and proliferative lesions of vascular endothelium are often demonstrated and may affect transport of oxygen and glucose through these structures. The longitudinal smooth muscle fibers in vein walls are hypertrophied in high-grade varicocele; however, it is not yet clear whether this is a cause or a consequence of varicocele. One can stipulate that vein wall hypertrophy may be the result of a high volume of blood flowing through the vessels or increased hydrostatic pressure. Most of the studies attempting to describe the changes in testicular histology associated with varicocele, documented the bilateral nature of these changes. Biopsy is not indicated in varicocele except in azoospermic patients in which it is predictive of the treatment outcome after varicocele repair.[32]

METHODS

Research strategy: A case control study conducted on twenty infertile patients with clinically evident varicocele; half of them were smokers. And a control group was formed of twenty fertile men without clinical varicocele and with normal semen parameters; half of them were smokers. The patients were randomly selected from the Andrology outpatient clinic of the Main University Hospital, Faculty of Medicine- University of Alexandria. The control group was randomly selected from other outpatient clinics of the same hospital.

Exclusion criteria: included Current or past history of occupational exposure to cadmium, Family history of infertility, History of testicular trauma, Urogenital infections with leukocytospermia (more than 1×10^6/ml) in early morning urine analysis and finally Patients suffering from abnormal epididymal consistency or induration of the vas deferens as revealed by genital examination.

Data were collected from all patients and their control after taking an informed written consent through interviewing schedule that included:

1. All studied persons were interviewed to collect data about personal, medical, occupational, smoking, family history of infertility and history of testicular trauma.
2. Clinical general and andrological examinations were conducted on all studied persons including

Inspection of Skin of the scrotum for the presence of visible dilated veins and examination of the penis including the location of the urethral meatus.

Palpation:

– Size of both testes was assessed as well as their consistency and position.

– Epididymis was palpated to estimate its dimensions, condition of the head, body and tail.

– Cord**:** the vas deferens was examined for consistency, presence of beading or indurations, and pampiniform plexus enlargement or engorgement to select clinically evident varicocele (grade II & III).

– Every patient was asked to perform valsalva maneouvre while the thumb and the index fingers of the examiner grasping the spermatic cord in the scrotum to detect change in size of veins.

– Presence of a thrill in scrotal veins when the patient is instructed to cough indicates venous reflux.

3. Semen analysis was conducted and assessed according to WHO criteria (1999).[33] At least two samples were obtained by masturbation after 2-7 days of sexual abstinence and the interval between two samples was not more than 3 weeks. The semen analysis was done using Computer-Assisted Sperm Analysis (CASA) technique.[34]

4. Metal in semen:

All polypropylene tubes and materials used (plastic and glass) were previously washed with 10% suprapure nitric acid overnight and thoroughly rinsed with deionized water. Sperms and seminal fluid were separated by centrifugation at (600 *g*, 10 min). The samples were frozen and stored at –20°C until analyzed using atomic absorption spectrophotometer. Semen samples were digested in 0.1 M HNO3 (one part sample to three parts HNO3). These proportions gave the most consistent results for semen analysis. Standard cadmium solutions in 0.1 M nitric acid were prepared and analyzed in the same manner as the samples. Cadmium was assayed by flame atomic absorption using the wavelength 248.3 nm cadmium line. This method was rapid and efficient. In order to avoid interference, Glassware and reagents were carefully checked for cadmium contamination by analyzing reagent blank. The measurement time was 3 sec. Calibration and standardization: a calibration curve was prepared by calculating the least square fit line using the standard concentrations and the analytical response. [35]

5. Scrotal Gray-scale and Color Doppler US were done for the studied infertile varicocele patients to detect varicocele grading.

Statistical measures used:

- Descriptive statistics including frequency, distribution, mean and deviation were used to describe different characteristics.
- Univariate analysis including T test and Person Chi Square test were used to test the significance of the result of the 5% level of significance.
- Mann-Whitney test of significance, Fisher's Exact test of significance, Likelihood Ratio were used for data that is not normally distributed.
- Partial correlation which was performed to measure the linear inter-relationship between cadmium exposure and seminal parameters and varicocele grading.

RESULTS

Personal characteristics:

Age of cases ranged between 21 and 42 years with a mean of 32.5±55.94 years. Meanwhile, it ranged from 22 to 41 years with a mean of 29.45±4.69 years among control group. Yet, the observed difference was statistically non significant (P= 0.08).

Smoking history (table I):

The smoking history revealed statistically non significant observed differences between smoking patients and smoking control regarding number of cigarettes smoked daily, duration of smoking and smoking index[148] (p= 0.66, 0.81 and 0.9 respectively).

Seminal parameters among the studied cases and control (II):

Sperm count among cases ranged between 0 and 20 million/ml with a mean of 7.87±7.53 million/ml. On the other hand, it ranged among control from 18 to 60 million/ml with a mean of 35.95±10.98 million/ml. Here, the observed difference was statistically significant (P< 0.0001). Sperm motility among cases ranged from 0 to 70% motile sperms with a mean of 14.7±16.36%. Meanwhile, among control it ranged between 40 and 70% motile sperms with a mean of 55.8±9.08%. The observed difference was statistically significant (P<0.0001). Sperm morphology among cases ranged between 10 and 90% abnormal forms with a mean of 46.6±28.89%. Meanwhile, among control it ranged from 20 to 40% abnormal forms with a mean of 31.1± 5.64%. Also, the observed difference was statistically significant (P= 0.02).

Seminal parameters among the studied patients; smokers and non smokers, (table III):

Sperm motility among smoking patients ranged from 0 to 30% motile sperms with a mean of 9.6±9.80%. While, among non smoking patients it ranged between 0 and 70% motile sperms with a mean of 19.8±20.28%. Yet, there was a statistically non significant observed difference (P= 0.19).

Seminal parameters among control; smokers and non smokers (table IV):

Sperm motility among smoking control ranged from 40 to 60% motile sperms with a mean of 49.9±6.78%. On the other hand, it ranged among non smoking control between 50 and 70% motile sperms with a mean of 61.7±7.11%. The observed difference was statistically significant (P= 0.003).

Cadmium level measured in seminal plasma among the studied subjects (table V):

Seminal plasma cadmium level of non smoking cases ranged between 1.15 and 1.48µg/dl with a mean of 1.34±0.1µg/dl. While, among non smoking control it ranged from 0.45 to 1.21µg/dl with a mean of 0.77±0.22µg/dl. The observed difference was statistically significant (P<0.001). Cadmium level in the seminal plasma of smoking control ranged from 0.92 to 1.22µg/dl with a mean of 1.08±9.59µg/dl. Meanwhile, among non smoking control it ranged between 0.45 and 1.21µg/dl with a mean of 0.77±0.22µg/dl. Also, the observed difference was statistically significant (P= 0.002). Furthermore, cadmium level in seminal plasma of the infertile smokers ranged between 1.48 and 7.28µg/dl with a mean of 3.24±2.25µg/dl. On the other hand, it ranged from 0.92 to 1.22µg/dl with a mean of 1.08±0.10µg/dl among the smoking control. The observed difference was statistically significant (P 0.001). Fig. 1

Cadmium level correlations with semen parameters & US grades:

- Partial correlation between average cadmium level and seminal parameters of the studied cases and control revealed that cadmium is correlated with sperms concentration and motility even after adjustment for smoking, non smoker/ even smoker (P= 0.009 and 0.003 respectively). Fig. 2
- Correlation between cadmium level in seminal plasma and seminal parameters revealed a statistically significant negative correlation between cadmium level and sperm morphology among cases (P= 0.003). Fig. 3
- Correlation between cadmium level in seminal plasma and seminal parameters among cases revealed that cadmium is negatively correlated with seminal volume and sperm morphology among the smokers (p= 0.02 and 0.03 respectively). Fig. 4
- Correlation between cadmium level in seminal plasma and seminal parameters among the smoking control revealed that cadmium is negatively correlated with sperms concentration (p= 0.02). Fig. 5
- Correlation between cadmium level in seminal plasma and US grading of varicocele among the studied patients revealed that cadmium level is positively correlated with US grades of varicocele among cases and also among both smoking and non smoking patients (P= 0.004, 0.001 and 0.04 respectively). Fig. 6

Table I: Smoking pattern among smoking cases and control.

Smoking pattern	Cases (n=10)	Control (n=10)	Test of significance
Number of cigarettes / day			Z= -0.43 P= 0.66
Min-Max	20-30	20-30	
Mean±SD	25.0±5.27	24.0±5.16	
Duration of smoking / year			Z= -0.23 P= 0.81
Min-Max	6-25	10-20	
Mean±SD	15.5±5.25	15.1±3.92	
Smoking index			Z= -0.11 P= 0.9
Min-Max	180-750	200-600	
Mean±SD	395.0±190.2	366.0±130.4	

Z. Mann-Whitney Test of Significance
*. Significant at P≤ 0.05

Table II: Seminal parameters among the studied cases and control.

Seminal parameters	Cases (n=20)	Control (n=20)	Test of significance
Concentration (million/ml)			T= 9.42 P<.0001*
Min-Max	0 - 22	18 - 60	
Mean±SD	7.87±7.53	35.95±10.98	
Motility (%)			T= 9.82 P<.0001*
Min-Max	0 - 70	40 - 70	
Mean±SD	14.7±16.36	55.8±9.08	
Morphology (%)			T= -2.35 P=0.02*
Min-Max	10 - 92	20 - 40	
Mean±SD	46.6±28.89	31.1±5.64	

Z. Mann-Whitney Test of Significance
*. Significant at P≤ 0.05

Table III: Seminal parameters among the studied smoking and non smoking patients.

Seminal parameters	Smoking patients (n=10)	Non smoking patients (n=10)	Test of significance
Concentration (million/ml)			Z= -1.97 P= 0.04*
Min-Max	0 -17	1.15 - 22	
Mean±SD	4.84±5.99	10.90±7.97	

Z. Mann-Whitney Test of Significance
*. Significant at P≤ 0.05

Table IV: Seminal parameters among control; smokers and non smokers.

Seminal parameters	Smoking control (n=10)	Non smoking control (n=10)	Test of significance
Motility (%)			Z=-2.96 P=0.003*
Min-Max	40.0-60.0	50.0-70.0	
Mean±SD	49.9±6.78	61.7±7.11	

Z. Mann-Whitney Test of Significance
*. Significant at P≤ 0.05

Table V: Cadmium level measured in seminal plasma among the studied subjects.

Variable	Cadmium level (µg/dl)			Test of significance
	n	Min-Max	Mean±SD	
Smoking cases	10	1.48 - 7.28	3.24±2.25	Z= 3.797*
Smoking control	10	0.92 - 1.22	1.08±0.10	P< 0.001*
Non smoking cases	10	1.15 - 1.48	1.34±0.1	Z= 3.705
Non smoking control	10	0.45 - 1.21	0.77±0.22	P< 0.001*
Smoking among control				Z= -3.1 P= 0.002*
Smokers	10	0.92 - 1.22	1.08±9.59	
Non smokers	10	0.45 - 1.21	0.77±0.22	

Z. Mann-Whitney Test of Significance
*. Significant at P≤ 0.05

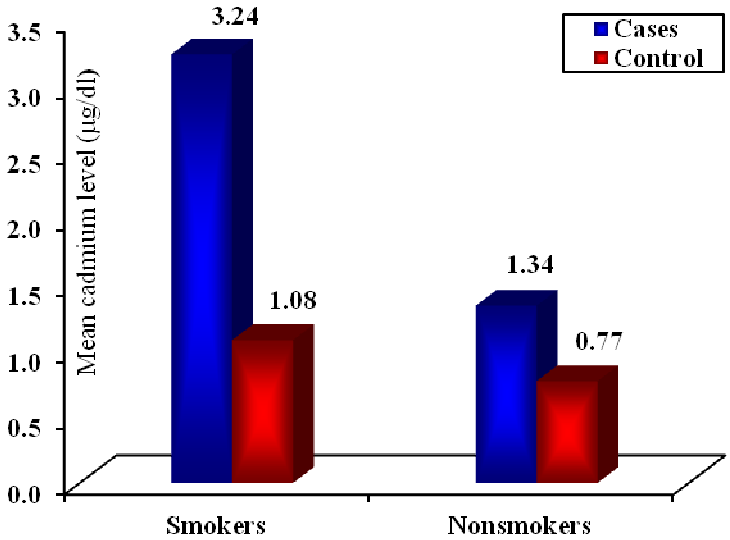

Fig 1: Cadmium level measured in seminal plasma among the studied subjects.

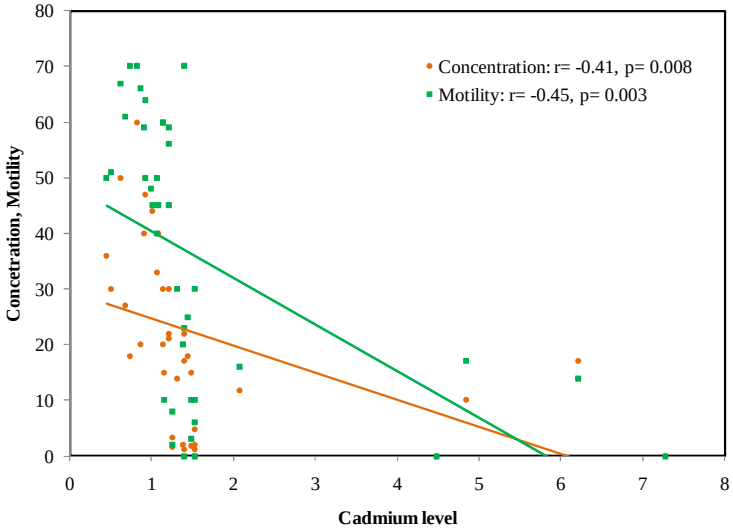

Fig 2: Negative correlation of cadmium level with both sperm count and motility among the studied persons.

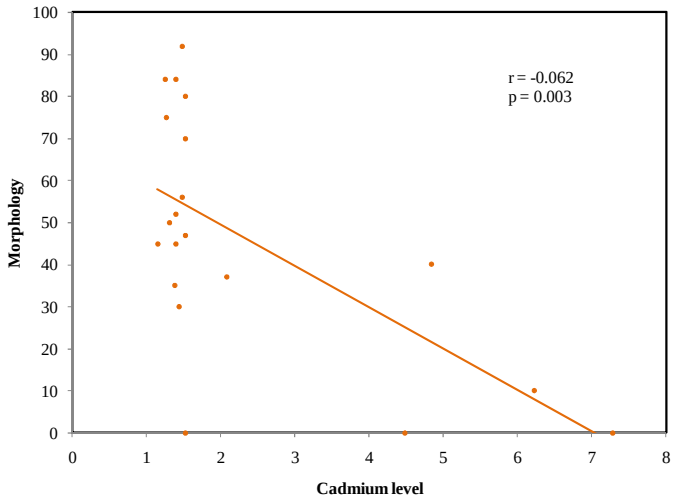

Fig 3: Negative correlation of cadmium level with sperm morphology among cases.

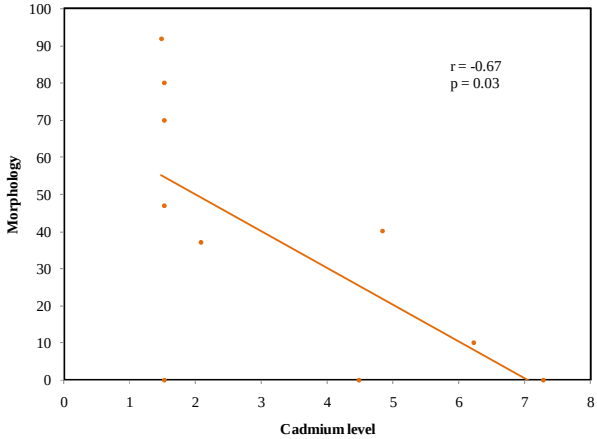

Fig 4: Negative correlation of cadmium level with sperm morphology among smoking cases.

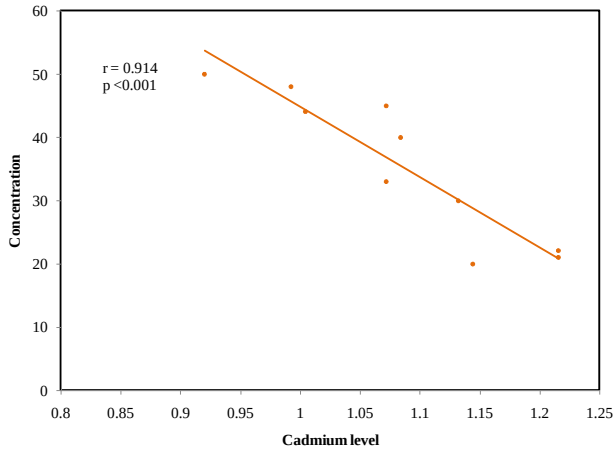

Fig 5: Negative correlation of cadmium level with sperm count among smoking control.

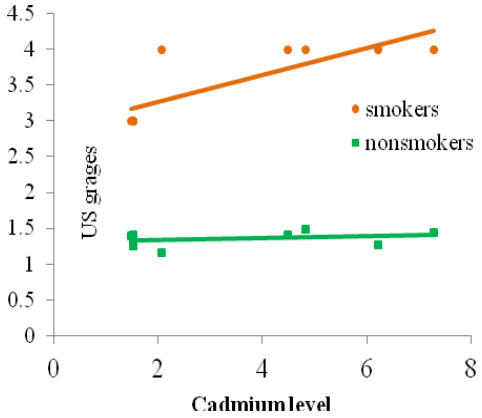

Fig 6: positive correlation of cadmium level with US grades among smoking and non smoking cases.

DISCUSSION

There is a debate in literatures about varicocele and male infertility.[5] The current study demonstrated statistically significant differences in seminal parameters between the studied cases and their control regarding decreased sperm concentration and motility, in addition to increased abnormal sperm morphology among cases (P= 0.0001, 0.0001 and 0.02 respectively).

These results came in accordance with many of other previous studies that demonstrated the detrimental effect of varicocele on sperm quality, for example; Zini et al, 2000, 2002[15, 36] demonstrated that varicocele is associated with stress sperm pattern in the form of increased number of abnormal forms, decreased progressive motility and decreased sperm density.

In addition, this association of varicocele with impaired seminal parameters came in accordance with Fábio et al, 2005[37] who demonstrated low sperm concentration and motility in infertile patients with varicocele as compared with their control. Also it came in accordance with Joong et al, 2007[38] who demonstrated that azoospermia or severe oligoathenospermia is associated with varicocele and patients with non obstructive azoospermia had sperms in their ejaculate and even the possibility of natural conception after microsurgical varicocelectomy. Also, Tamer et al, 2009[39] demonstrated that patients with non obstructive azoospermia had motile sperms via ejaculate and even the chance of natural conception after High ligation varicocelectomy.

By comparing smoking to non smoking cases, the current study revealed a statistically significant observed difference regarding decreased sperm concentration among the smokers (p=0.04). Meanwhile, by comparing smoking with non smoking control this study revealed a statistically significant observed difference regarding decreased sperm motility among the smokers (P= 0.003). Many authors demonstrated the negative impact of cigarette smoking and the exposure to cadmium on sperm parameters. For example; Kumosani et al, 2008[11] demonstrated statistically significant

associations between cigarette smoking and round head sperm morphology and decreased both sperm count and motility in a study conducted on 70 infertile smoking and non smoking patients. Also, Mack et al, 2000[40] demonstrated the detrimental effect of cigarette smoking on sperm quality; most significantly sperm concentration, motility, and morphology. In addition, Robert et al, 2003[41] demonstrated that smoking is associated with impaired semen quality in infertile smokers. On the other hands, Ramadan et al, 2002[12] in a previous study demonstrated non significant differences in standard sperm parameters (concentration, motility, and morphology) between infertile smokers and nonsmokers.

The present study demonstrated a statistically significant high level of cadmium in seminal plasma of the studied patients with varicocele as compared to their control ($p<0.0001$). Precisely, cadmium level was significantly high in seminal plasma of studied infertile smokers suffering from varicocele in comparison to their smoking control in spite of absence of significant difference between neither number of cigarettes smoked daily nor duration of smoking ($p< 0.001$). Consistently, cadmium level in seminal plasma of studied infertile non smokers suffering from varicocele was significantly higher than that measured among their non smoking control ($p< 0.001$). Thus, varicocele causes accumulation of cadmium in seminal plasma as compared to normal subjects. Since half of the studied patients were non smokers, as well as the occupational exposure to cadmium was excluded from this study, so the detected high cadmium level in seminal plasma from those patients can't be explained except by environmental exposure.

Benoff et al, 2004[8] and Hyeon et al, 2005[17] demonstrated that cadmium is higher in seminal plasma from infertile men with varicocele than those without varicocele. In addition, they demonstrated that cadmium level in testicular biopsy from infertile non smoking men with varicocele was higher than their control subjects with obstructive azoospermia. Moreover, Hyeon et al, 2005[17] demonstrated that the testis in varicocele lacks an active pump for removing cadmium, so, over time, testicular cadmium level can be accumulated.

Furthermore, Benoff et al, 1997[5] and Erica et al, 2009[6] demonstrated that infertile men with varicocele have high levels of cadmium in their seminal plasma as a result of environmental cadmium exposure. They also demonstrated that cadmium is toxic to reproductive organs at levels appreciably lower than those required for toxicity by other organs. Testes are more sensitive to cadmium than other organs because of their unique vasculature. Moreover, disruption of the blood-testis barrier (BTB) is the major target of cadmium toxicity in the testis, and the underlying mechanism of action, Cadmium also accumulates in the prostate, epididymis and seminal vesicles, where a long biologic half-life is observed, They also suggested that susceptibility to cadmium induced testicular damage has been described and attributed to gene defect. So, it is possible that infertile men with varicocele express a similar gene that renders them more susceptible to cadmium in their environment.

The current study demonstrated a statistically significant high cadmium level in seminal plasma of infertile smokers in comparison with infertile non smokers ($p< 0.0001$). In addition, the present study also demonstrated that level of cadmium is high in seminal plasma from smoking control as compared with non smoking control ($P= 0.002$). Several studies demonstrated that cigarette smoking is associated with accumulation of cadmium in the testis. Benoff et al, 2000[42] and Erica et at, 2009[6] demonstrated that cigarette smoking adversely affects varicocele-associated infertility by increasing the level of cadmium in seminal plasma. Similarly, Kumosani et al, 2008[11] demonstrated that cadmium level is significantly higher in seminal plasma of smokers than non smokers, in a study conducted on 159 semen samples (61 smokers and 98 non smokers). Moreover, Benoff et al, 1997[5] demonstrated that absorption of cadmium by the lungs is two-fold greater than that by the gastrointestinal tract. Thus, the greatest danger is from inhalation of environmental aerosols that are present in cigarette smoke, which explain presence of high cadmium level in seminal plasma among infertile smokers with varicocele than infertile non smokers with varicocele.

The present study revealed that cadmium is correlated negatively with sperm concentration and motility among the studied patients and their control even after adjustment for smoking, non smoker/ even smoker, ($P= 0.009$ and 0.003 respectively). Many authors demonstrated the association of cadmium accumulation in the testes with impairment of semen quality. Benoff et al, 2000[42] and Benoff et al, 2004[8] demonstrated the presence of high level of cadmium in seminal plasma among infertile patients with varicocele and its significant correlation with high number apoptotic cells within the seminiferous tubules. Similarly, Yin et al, 1997[23] and Benoff et al, 1997[5] demonstrated that the accumulation of toxic agents including the products of cigarette smoke such as cadmium is one of the causes of apoptosis in varicocele.

Furthermore, Hyeon et al, 2005[17] demonstrated that patients with varicocele have significant increase in spermatozoal DNA damage, which is attributed to high ROS level in semen. The overproduction of ROS is crucial as an apoptosis-triggering mechanism. Thus, they suggested that

apoptosis is the cause of oligospermia among men with varicocele. Moreover, Fujisawa et al, 2003[43] found that the concentration of the soluble form of Fas (s-Fas), which block Fas-dependent apoptosis, in seminal plasma of oligospermic men with varicocele was significantly lower than in oligospermic men without varicocele and their normal control. This reduced s-Fas level was reversed by varicocelectomy.

The current study revealed that cadmium level in seminal plasma is correlated with sperm morphology among the studied infertile patients with varicocele and also among the infertile smokers (P= 0.003). The correlation between cadmium and sperm morphology was demonstrated by many authors, for example; Ming et al, 2008[44] demonstrated, in a previous study conducted on 341 men, that environmental accumulation of cadmium in semen reduces sperm quality; mostly sperm morphology. In addition, Erica et at, 2009[6] also demonstrated that the exposure to cadmium is an inducer of testicular oxidative stress by markedly reducing the testicular antioxidants enzymes, such as superoxide dismutase, catalase and gluthathione peroxidase. Furthermore Benoff et al, 2004[8] suggested that cadmium is associated with the stress sperm morphology and the acrosome reaction insufficiency typical seen in men with varicocele. They also demonstrated that cadmium disrupts the cytoplasmic actin cytoskeleton, which in fertile men contributes to shaping of the acrosome around the sperm nucleus and to effect acrosome exocytosis. So, that correlation between cadmium and sperm morphology could be explained.

Conclusions

- Varicocele is associated with impairment of seminal parameters including decrease in sperms concentration and motility as well as increase in abnormal sperm morphology.
- Environmental exposure to cadmium leads to its accumulation in seminal plasma of patients with varicocele-associated infertility as compared with normal males.
- Cigarette smoking results in accumulation of cadmium in seminal plasma of smokers especially among patients suffering from varicocele.
- Cadmium causes decrease in sperm count and motility with an increase in the number of sperms depicting abnormal morphology.

Suggested recommendations:

Cadmium level measurement in seminal plasma should be used as a routine investigation for infertile varicocele patients, Health education about hazards of smoking and heavy metals on male fertility should be included in health education programs, and Further research studies on therapeutic modalities to block pathogenesis of Cadmium-induced male infertility; such as MAPK-signaling pathway, ROS and apoptosis, could be future recommendations.

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