ORIGINAL ARTICLE

NEURO PHYSIOLOGICAL ROLE OF SILDENAFIL CITRATE (VIAGRA™) ON SEMINAL PARAMETERS IN DIABETIC MALES WITH AND WITHOUT NEUROPATHY

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ABSTRACT

Sildenafil citrate is a specific inhibitor of phosphodiesterase (PDE) type-5 and represents a powerful therapy for male erectile and fertility dysfunctions of different etiologies. Present study demonstrates whether sildenafil administration modifies seminal parameters in diabetic neuropathic patients.

In this investigation 50 insulin dependent (IDDM) and 50 non insulin dependent (NIDDM) diabetic male patients with and without an objective evidence of neuropathy and 50 age matched non diabetic male controls were selected. Every male had age between 20 to 65 years with duration of diabetes distributed over 1 to 20 years. Treatment with 100 mg of oral sildenafil citrate on seminal parameters was evaluated by semen analysis in these patients. In both IDDM and NIDDM diabetic neuropathic patients, chronic sildenafil treatment exhibited a significant decrease in total sperm output and sperm concentration ($p<0.001$). On the other hand, sperm motility and semen volume were found to be increased by about 40% and 48% respectively in these patients, where as sperm morphology and quality of sperm motility remained unaffected. However both types of non neuropathic diabetics showed a non significant difference in all the above mentioned parameters when compared with the untreated groups and their respective control subjects. A comparison between IDDM and NIDDM neuropathic and non neuropathic diabetic groups further indicated a non significant difference in all the parameters of semen analysis.

These findings suggest a chronic neuro physiological effect of sildenafil treatment on male fertility profile exclusively in diabetic neuropathic condition with an improvement in testicular function which was probably arrested due to some kind of testicular hyperplasia resulted by testicular necrosis and promoted spermatogenesis. Sildenafil seems to be associated with an improvement in the entire smooth musculature of reproductive tract and testicular morphology which was altered due to neuropathy like a reduction in excess accumulation of interstitial collagen and calcification in the smooth muscles of seminiferous tubules which made them rigid leading to atonia of bladder and urethra which resulted in partial or retrograde ejaculation associated with a decreased sperm motility. Sildenafil treatment returned back the spermatogenesis to normal with a positive influence on sperm motility and ejaculate volume in these neuropathic patients irrespective of the type of diabetes.

Keywords: Diabetes; neuropathy; semen; sildenafil citrate therapy.

INTRODUCTION

Direct evidence for a neuropathic etiology of diabetic erectile dysfunction comes from studies that show structural changes in autonomic nerve fibers supplying the corpora cavernosa (Ellenberg and Weber, 1966). Emission disturbances that occur in diabetes are associated with involvement of the sympathetic fibers that sub serve the seminal vesicle, vas deferens and bladder trigone (Brown *et al*., 2006). Testicular anesthesia, presence of a neurogenic bladder and a delayed bulbocavernous reflex latency are indirect evidence for a neuropathic etiology of patient's complaints. Failure of ejaculation secondary to emission disturbances due to sympathetic denervation of the vas deferens is another manifestation of autonomic neuropathy, usually seen in more advanced stage (Crick and Lequier, 1978).

Abnormalities of spermatogenesis, sperm count, motility and seminal fluid volume have been offered in support of an endocrine basis for impotence in diabetes (Klebano and Macleod 1960; Jackson, 2004; Scarano *et al*., 2006). Instances of complete absence of sperm are most readily explained by retrograde ejaculation (Ellenberg and Weber, 1966). Motility disturbances of sperm have previously been observed in diabetics with autonomic neuropathy (Bartak *et al*., 1975). Biopsy specimens have been reported to show the thickening of the basement membrane of the seminiferous tubules and testicular tissues, a diminution in the number of Leydig cells and abnormal spermatogenesis (Guvel *et al*., 2004).

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In contrast to the extensive literature on the reproductive capacity of diabetic neuropathy, relatively little has been reported on the influence of diabetes upon semen quality. Males with juvenile diabetes frequently show a lag in sexual development, a decrease in libido and potential and erectile impotence (25%). Similarly, the excretion of gonadotropin has been studied. Hertzman, (1973) did not find any elevation, but Ando *et al*. (1984) showed that excretion of gonadotropin was diminished in diabetic neuropathic males. Apparently, no observations on the semen were made in these studies, but in six of these individuals, occasional to many spermatozoa were found in the urine immediately after orgasm. Neuropathy seemed more prevalent than any measured complication of diabetes in these patients.

In spite of the advancing knowledge in recent years regarding the nature and treatment of diabetes, including its complications, subject of diabetic neuropathy with special reference to fertility parameters has become more confused and is still probably one of the most common complication in diabetics, and yet it remains poorly understood and inadequately explored (Ando *et al*., 1984).

One interesting new breakthrough in the treatment of erectile dysfunction of different etiology using oral drugs lies in the substance sildenafil (Viagra™) seems to be a most promising discovery (Karim *et al*., 2006). Recently, sildenafil has been shown to restore erections in temporary erectile dysfunction related to the need of semen collection for assisted reproductive techniques.

Sildenafil is a potent and selective inhibitor of the cyclic guanosine monophosphate (cGMP)-specific phospho-diesterase type 5 (PDE_5), which is responsible for the degradation of cGMP in the corpus cavernosum (Goldstein *et al*., 2002; Fujiswa and Sawada, 2004). It has a peripheral site of action on erection. It potently enhances the relaxant effect of nitric oxide (NO) on this tissue. When the NO/cGMP pathway is activated, as occurs with sexual stimulation, inhibition of PDE_5 by sildenafil results in increased corpus cavernosum levels of cGMP.

Nine different PDE isoenzymes (PDE_1 to PDE_9) have been described and found to be present at various concentrations in human tissues (Fabbri *et al*., 1999).

Previous studies have shown that mRNA coding for cAMP-specific PDE (PDE_4A) isoforms are present in mature rat and mouse germ cells (Naro *et al*., 1996) and that the expression of these isoforms is maximal in round spermatids and is maintained in mature spermatozoa (Soderling *et al*., 1998b). Nitric oxide synthase (Lewis *et al*., 1996) and two distinct PDE isoforms (PDE_1 and PDE_4) are present in human sperm cells (Fisch *et al*., 1998). Sildenafil is a specific and potent inhibitor of cGMP-specific phosphodiesterase (PDE) type-5, which is the predominant PDE isoenzyme responsible for the degradation of cGMP in the corpus cavernosum, and has also minor inhibitory effects on PDE_6 and PDE_1 activities (IC_{50} = 0.034 and 0.28 μmol/l respectively) (Morales *et al*., 1998). It has recently been shown that some degree of erectile dysfunction may be present in male infertile partner and these men have difficulties in producing spermatozoa at the time of egg fertilization (Tur-Kapsa *et al*., 1999). This fertility dysfunction can be successfully treated with sildenafil.

It has been demonstrated that human sperm cells contain as yet uncharacterized PDE isoforms which are different from PDE_1 and PDE_4, and that the in-vitro inhibition of sperm PDE_1 and PDE_4 isoenzymes by specific inhibitors stimulates acrosome reaction and sperm motility (Fisch *et al*., 1998). Also, it is known that after acute administration of 100 mg sildenafil, the drug reaches a concentration of 0.1-0.3 μmol/l in the ejaculate (Pfizer, Viagra™ data sheet). This concentration is consistent with a possible inhibitory interaction of sildenafil with sperm PDE isoforms (Fabbri *et al*., 1999). Furthermore, treatment with sildenafil is well tolerated and is associated with minimal adverse events (e.g., headache, flushing, and dyspepsia) that rarely cause discontinuation of treatment (Park, 2005).

Our current work is designed to study the effect of sildenafil citrate oral administration (100 mg) on seminal parameters in diabetic neuropathic men to restore fertility.

MATERIALS AND METHODS

For experimental purposes and for the studies of diabetic neuropathy, 50 insulin dependent (IDDM) and 50 non insulin dependent (NIDDM) diabetic male patients with and without an objective evidence of neuropathy and 50 age matched non diabetic male controls were selected. Every male aged between 20 to 65 years with duration of the onset of the disease from 1 to 20 years was included.

The presences of diabetic complications were assessed by a review of the medical record. Neuropathy was present if the records indicated absence of ankle jerk, decreased vibration sense or pin prick sensation in the feet or hands, or there was history of neuropathic pain, foot ulcer, or symptoms compatible with autonomic neuropathy (differential diagnosis) including postural hypotension, intermittent diarrhea especially nocturnally, epigastric fullness, bladder dysfunction, diminished sweating in the legs, gustatory sweating and hypoglycemic unawareness. The criteria for the presence of symptomatic autonomic neuropathy were two or more severe or three or more mild/moderate features.

Impotence was determined according to the methods

described previously (Bancroft and Bell, 1985; Rosen *et al*., 1997). Men were considered candidates for this study when they had complained of erectile dysfunction with diabetic neuropathy for 6 or more months.

Diabetic treatment was recorded as di*et al*one, oral hypoglycemic agent or insulin. Inquiry was made of other drug therapy, angina pectoris, previous myocardial infarction or cardiac failure, intermittent claudication, thyroid dysfunction, previous sympathectomy or other abnormality that might predispose to organic impotence such as neurological disease or previous injury.

To assess the efficacy and safety of oral sildenafil citrate (Viagra™-Pfizer, USA) in the treatment of fertility dysfunctions in diabetic men with and without neuropathy, subjects from home and clinical practice centers in the local vicinities, were randomized to receive sildenafil citrate (100 mg orally), but not more than once daily, for 12 months. All subjects enrolled in the study after giving an informed consent to the study protocol, which was approved by the ethical committee. Self-reported ability to achieve and maintain an erection for sexual intercourse according to the International Index of Erectile Function and adverse events were recorded according to the method described previously (Rosen *et al*., 1997).

During treatment with drug, semen specimens were collected by masturbation one hour after sildenafil consumption (intake: 3 hours after the meal approximately) and were evaluated for total sperm output, sperm concentration, sperm motility, semen volume, sperm morphology (oval percentage) and quality of motility using microscopic examination (scale ranging 0-4) according to World Health Organization guidelines (WHO, 1992).

Statistical analysis was done by tabulating the frequency distribution for six semen parameters: (1) total sperm output; (2) sperm concentration; (3) semen volume; (4) percent motile; (5) quality of motility; and (6) percent oval forms. Based on the results of the frequency distribution and clinical judgment, boundaries were assigned to categorize each of the six parameters as either poor, equivocal, or good. Student *t*-test for the significant differences between means of treated/untreated neuropathic and non-neuropathic men was performed on all six parameters. In all instances probability ($p<0.05$) was regarded as statistically significant. The Azoospermic cases were excluded from this data.

RESULTS

The data for the estimated values of total sperm output and sperm concentration before and after the administration of the 100 mg. of oral dose of sildenafil citrate in 50 IDDM and 50 NIDDM diabetic men (with and without neuropathy) and in 50 age matched non-diabetic controls are shown in figures 1 and 2. The values of total sperm output and sperm concentration estimated from sildenafil treated diabetic neuropathic patients compared with the values obtained from untreated patients showed a highly significant difference ($p<0.001$). This difference showed about 63% and 45% decrease respectively being almost equal to the values of the control subjects.

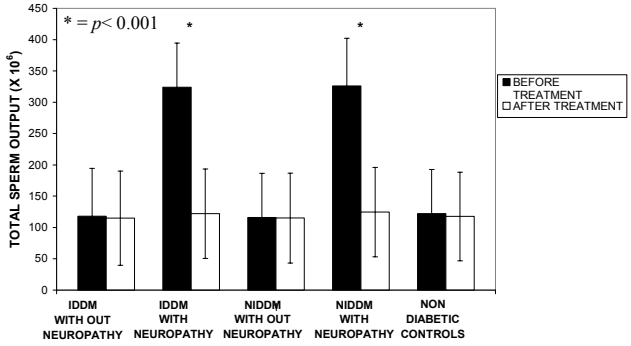

Fig. 1: Total sperm output before and after oral administration of sildenafil citrate (100 mg dose) in (IDDM) and (NIDDM) diabetic males (with and without neuropathy) and control subjects. Values are means ± S.D.

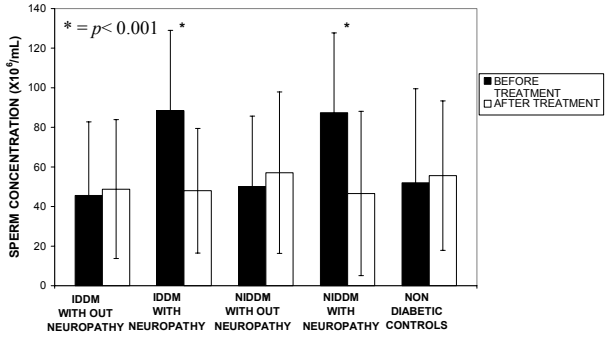

Fig. 2: Sperm concentration before and after oral administration of sildenafil citrate (100 mg dose) in (IDDM) and (NIDDM) diabetic males (with and without neuropathy) and control subjects. Values are means ± S.D.

However, this difference was found to be non-significant in both IDDM and NIDDM patients without neuropathy before and after the oral administration of drug, and when compared with their respective controls of same age group. In contrast an inverse relationship was found when sperm motility (%) and semen volume were studied

in the same specimens. Both types of treated diabetic neuropathics showed a significant increase ($p<0.005$) in the values of sperm motility (fig. 3) and comparatively more significant increase ($P<0.001$) in the values of semen volume (figure 4). However, both types of treated diabetic patients without neuropathy showed a non significant difference in both the above mentioned parameters when compared with the untreated patients and their age matched control subjects. A non significant difference was observed when the sperm morphology and the quality of motility was determined either in both IDDM and NIDDM treated or non treated neuropathic/non neuropathic diabetic patients and their age matched non diabetic control subjects (figs. 5 and 6). In addition no difference was observed in IDDM and NIDDM values when compared with each other either before or after the oral administration of sildenafil in all the groups (data not shown).

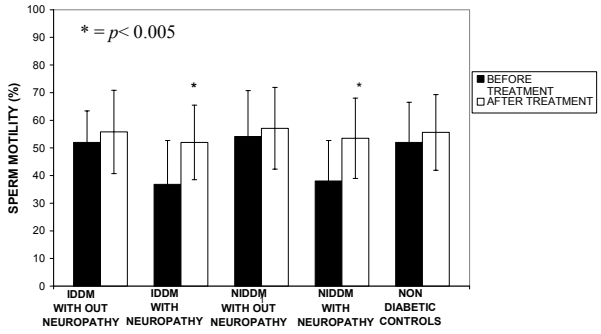

Fig. 3: Sperm motility before and after oral administration of sildenafil citrate (100 mg dose) in (IDDM) and (NIDDM) diabetic males (with and without neuropathy) and control subjects. Values are means ± S.D.

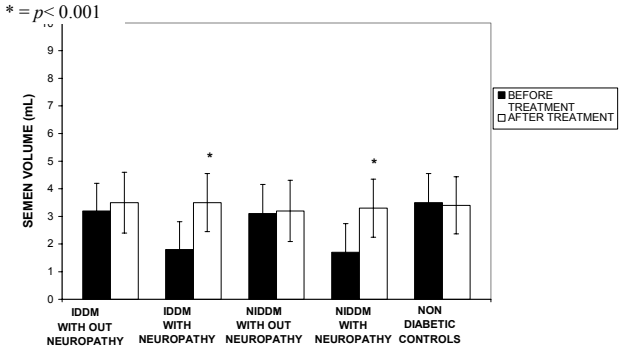

Fig. 4: Semen volume before and after oral administration of sildenafil citrate (100 mg dose) in (IDDM) and (NIDDM) diabetic males (with and without neuropathy) and control subjects. Values are means ± S.D.

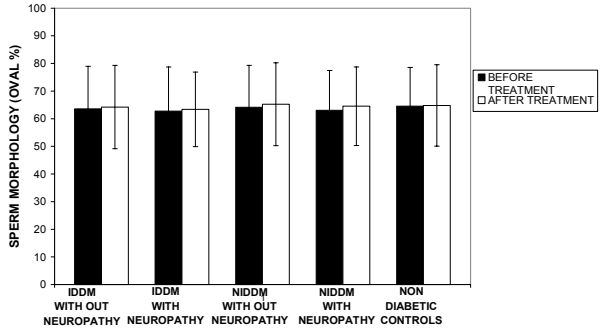

Fig. 5: Sperm morphology before and after oral administration of sildenafil citrate (100 mg dose) in (IDDM) and (NIDDM) diabetic males (with and without neuropathy) and control subjects. Values are means ± S.D.

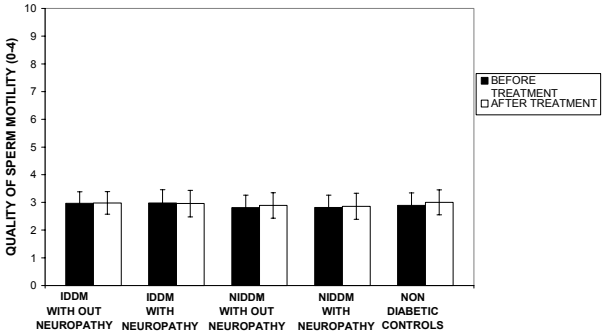

Fig. 6: Quality of sperm motility before and after oral administration of sildenafil citrate (100 mg dose) in (IDDM) and (NIDDM) diabetic males (with and without neuropathy) and control subjects. Values are means ± S.D.

DISCUSSION

Limited information is available for humans to test the biological fertility potential of semen. Diabetic neuropathy in particular is more neglected area regarding the semen analysis and almost no data is available with regard to the clinical management of this fertility parameter. Therefore, in the present study physiological changes have been investigated in semen components in IDDM and NIDDM diabetics (with and without neuropathy) and the possible effect of sildenafil citrate on seminal parameters. Recently, sildenafil (Viagra^{TM}) is being used as a quality drug for the management of men with erectile and fertility dysfunctions and a breakthrough compared with previously available treatments, e.g., intracavernosal and intraurethral prostaglandin therapies, vacuum devices or penile implants. In fact, oral therapy always represents a more suitable option for all men

suffering from erectile and fertility disturbances and for the couple in the long term follow-up (Fabbri *et al*., 1999).

Our previous investigations (Ali *et al*., 1993) as well as those of others (Rubin and Babbott etal; 1985) demonstrate that sexual disturbances in male diabetics are common. When a reasonably normal ejaculate volume is forthcoming in the diabetic men there appear to be disturbances in spermatogenesis. These pathologic disturbances of potency may occur at any time after the manifestation of diabetes and are more frequent after the establishment of autonomic neuropathy. Associated with impotence a decrease in the size and consistency of testis and prostate in the diabetic men than normal have been reported (Schoffling *et al*., 1963). In addition, low sperm count with an increased percentage of non-motile or dead sperm have also been reported. Klebanow and McLeod., (1960) however did not observe a reduction in sperm count although they did noticed decrease sperm motility in 50% of the patients. They also observed 18 of the 36 diabetics with testicular dysfunction and reduced or absence of sexual potency and androgen deficiency, as indicated by low concentration of fructose in the semen. In addition, characteristic pathological changes accompanying reduced testicular activity were found in diabetic neuropathic patients (Ali *et al*., 1993). Low or absence of pituitary gonadotropin in urine and abnormality in gonadal morphology, with thickening of basement membrane of tubules, defective spermato-genesis, and reduced number of leydig cells were also found in these patients (Guvel *et al*., 2004). Where as these observations are consistent with hypogonadotropic hypogonadism, steroids investigations gave seemingly consistent results, i.e., increased urinary excretion of certain steroid metabolites (Morales, 1998).

The very limited data available on the effects of sildenafil citrate on seminal parameters reveals that the oral administration of sildenafil citrate has some role on seminal parameters in the patients of erectile dysfunction. At least two distinct PDE isoforms (PDE_1 and PDE_4) have been demonstrated to be present in human sperm cells. Specific inhibition of PDE_1 and PDE_4 by 8-methoxy-isobutyl-methylxanthine and rolipram selectively stimulates the Acrosome reaction and sperm motility respectively (Fisch *et al*., 1998). After assimilation sildenafil circulates in plasma at micro molar concentrations which can cause a minor inhibition of PDE_6 and PDE_1 activities (ED_{50} = 1:10 and 1:100 of PDE_5 inhibition respectively) and determine transient side-effects (Morales *et al*., 1998). In our study the maximum therapeutic dose of sildenafil was administered (100 mg) in order to achieve maximal drug concentration in seminal fluid and to investigate eventual sildenafil-spermatozoa interaction. It has been reported that in healthy volunteers, after acute 100 mg oral administration, sildenafil is present in seminal fluid in a concentration range of 0.1-0.3 µmol/l (Pfizer, Viagra^{TM} data sheet). At this concentration sildenafil has a potential to interact with PDE_1 and perhaps with other as yet uncharacterized PDE isoforms (30-40% out of total PDE activity) present in sperm cells (Fisch *et al*., 1998; Fabbri *et al*., 1999).

In the present study we have described here, probably for the first time, the effects of sildenafil citrate treatment (100 mg) on seminal parameters in both IDDM and NIDDM diabetic neuropathic patients with proven infertility.

Our results showed a significant decrease i.e., about 63% and 45% in total sperm out put and sperm concentration respectively in both IDDM and NIDDM diabetic neuropathic patients after the oral administration of sildenafil when compared with the same values obtained before the administration of drug. However this difference was found to be non-significant in both types of diabetic patients without neuropathy before and after oral administration of sildenafil, and when compared with their respective control subjects.

The values of sperm output and sperm concentration were found to be almost all similar when compared within non-diabetic control subjects before and after oral administration of sildenafil citrate.

These findings indicate the presence of chronic effects of sildenafil treatment on the male fertility profile exclusively in diabetic neuropathic condition with an improvement in testicular function which was probably arrested due to some kind of testicular hyperplasia resulted by testicular necrosis and promoted spermatogenesis in these diabetic patients as a result of autonomic neuropathy. Sildenafil treatment returned back the spermatogenesis to its normal. Similar suggestions have been made previously in non-diabetic patients with impotence (Tur-Kasp *et al*., 1999).

In contrast to sperm count and concentration, our results indicated about 40% and 48% increase in sperm motility and semen volume respectively in both IDDM and NIDDM diabetic neuropathic sildenafil treated patients than non neuropathic sildenafil treated diabetic men and their age-matched non diabetic controls.

In a recent study on in-vitro effects of sildenafil citrate, used for erectile dysfunction, on human sperm motility has shown that 200-microgram/mL dose of sildenafil citrate has no effect on sperm motility. However, the high dose (2000-microg/mL) significantly increase the nitric oxide synthase and nitrite production in human spermatozoa thus providing evidence that endogenous nitric oxide is beneficial to sperm motility (Lewis *et al*., 1996; Agarwal *et al*., 2006). These results are in

conformity with our present findings where in both IDDM and NIDDM diabetic neuropathic men sildenafil treatment improved the sperm motility.

A significant increase in semen volume in IDDM and NIDDM sildenafil treated diabetic neuropathic patients in our results indicates possible improvement in the autonomic/pathological lesion in the entire smooth musculature of reproductive tract and some kind of morphological alterations in the testis like a reduction in excess accumulation of interstitial collagen and calcification in the smooth muscles of seminiferous tubules which made them rigid leading to atonia of bladder and urethra which resulted in partial or retrograde ejaculation due to neuropathy.

In conclusion, our results indicate that in both IDDM and NIDDM non-neuropathic diabetics and in non-diabetic control subjects, chronic sildenafil citrate treatment does not modify semen characteristics and has a positive neuro physiological influence over the resumption of seminal functions and fertility capacities following neuropathy.

Our work reported in this research thus provides strong evidence of using sildenafil citrate in a diabetic neuropathic population for the purpose of restoring sexual functions. Further, sildenafil be further investigated as a new class of autonomically acting drug.

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