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RESEARCH ARTICLE

GONADAL HORMONE PROFILE AND SEMEN QUALITY ASSESSMENT OF THE DIABETIC PATIENTS UNDER REGULAR AND IRREGULAR TREATMENT MODE IN THE AGE GROUP OF 25-40 YEARS: A DURATION DEPENDENT COMPARATIVE APPROACH

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ABSTRACT

The present study was conducted to investigate the duration dependent changes in gonadal hormone and semen quality of the diabetic patients under regular and irregular treatment mode. In these aspect blood glycemic sensors, gonadal hormone profile, oxidative stress sensors in sperm pellet were assessed. Spermatological profiles were evaluated for testing the semen quality. Levels of blood glycemic sensors, gonadal hormones and values of spermatological parameters were corrected in diabetic patients under regular treatment mode for 4 to 5 years upto a certain and significant levels but were not changed further along with extension of the duration of the said treatment upto 15 years. But in case of irregular treatment the values of these parameters were changed towards the pathological direction further along with the extension of the duration of the treatment in respect to 4 to 5 years of treatment suffering from diabetic. Serum insulin and C-peptide were increased significantly in diabetic patient under regular treatment mode for 4 to 5 years in respect to control but these levels were stable when duration of the treatment was increased further. In case of diabetic patient under irregular treatment mode these levels were decreased along with extension of the duration of the treatment. Oxidative stress in sperm was increased in diabetic patient under irregular treatment mode in respect to diabetic patients under regular treatment mode in duration dependent manner. Though, oxidative stress in sperm showed a tendency to be lowered in diabetic patient who received regular treatment mode for 4 to 5 years in respect to irregular treatment, but all the levels of the sensors come stable when that duration of the treatment mode was extended beyond 4 to 5 years upto 15 years. So it may be concluded that regular treatment is more beneficial for recovery of testicular hypofunction in diabetic patients in respect to irregular treatment. Moreover, irregular treatment mode may exerts more deleterious effect on testicular physiology in diabetic patients along with extension of this treatment habit.

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INTRODUCTION

Diabetes mellitus is one of the oldest known human diseases whose devastating affects not only increasing day by day but severity is also almost in epidemic level. It has a disadvantageous effect on sexual function especially in male individuals (Rehman *et al*., 2001). Andrological changes have been reported in individuals with diabetes (Ballester *et al*., 2004). Sperm motility and viability have been decreased along with elevation in abnormal morphology of sperm and with low level of serum testosterone in diabetes which may lead to subfertility or infertility (Dinulovic and Radonic, 1990; Kodama *et al*., 1996). It has also been reported that small amount of reactive oxygen species (ROS) are essential for enhancing the fertilizing capabilities of spermatozoa (Kodama *et al*., 1996). So, the physiology of human spermatozoa needs oxidation-reduction processes for hyperactivation, capacitation

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and acrosome reaction (Sharma and Agarwal, 1996; Aitken, 1997). But excess amount of ROS generation may lead to severe oligospermia (Aitken *et al*., 1992). Oxidation of lipids, proteins and DNA are increased with the development of diabetes. Diabetic condition produces oxidative stress in the reproductive organs directly or indirectly by modulating the endocrine functions that results infertile condition. In diabetic condition seminal fructose level is also increased as fructose utilization by the sperm is decreases (Kodama *et al*., 1996). All above facts suggest the need for a large-scale epidemiological study and for further investigation about the relationship between male fertility and diabetes mellitus. Though, there are several researchers are working in this line focusing the diabetes induced male gonadal hypofuntion but very few studies have yet been conducted in human model in a duration dependent manner about the changes in testicular physiology in relation to diabetes. In addition, there is no report about the duration dependent effect of regular and irregular treatment mode on testicular physiology in diabetic patients at their peak reproductive age i.e. 25 to 40 years age group.

MATERIALS AND METHODS

Experimental selection

The present study was conducted in the Department of Gynecology and Obstetris, ESIC-PGIMSR, ESIC Hospital, Joka, Kolkata and Department of Bio-Medical Laboratory Science and Management, Vidyasagar University, Midnapore, West Bengal. The present study consists of 450 cases of chronic diabetes under regular treatment and 450 cases of chronic diabetic under irregular treatment with infertility complication in the age of 25 to 40 years. The values were compared with the values of 150 apparently healthy non-diabetics which were in the same age group. All the patients were from department of Gynecology and Obstetris, ESIC-PGIMSR, ESIC Hospital, Joka, Kolkata, who attended for checkups and treatment. Blood samples were obtained from vene puncture from antecubital vein of upper limb on the test groups. Anticoagulated whole blood was analysed for glycated haemoglobin i.e. HbA1C. Serum was separated from other part of the blood and analyzed by using standard methods. Semen samples were obtained after a recommended 2 to 5 days of sexual abstinence. All samples were subjected to a conventional light microscopic semen analysis to determine liquefaction, semen volume, sperm concentration and motility according to WHO recommendations (WHO, 1999). Semen analysis was performed within 1 h of ejaculation, following a period of incubation at 37°C to allow for liquefaction. The remaining semen was divided into aliquots and incubated at 37°C in preparation for further biochemical analysis. All subjects gave written informed consent for participation in this study, and the work was approved by the Institutional Ethics Committee of ESIC Hospital, Joka, Kolkata.

Patients selection

Patients who are diabetic under regular treatment or diabetes under irregular treatment were included in the study. On the other hand, the patient who are nondiabetic but infertile, and those who are diabetes but beyond the age of 40 years were excluded from the study. Male diabetic patients with testicular hypofunction or not came through outdoor patient department (OPD) of Dept. of Gynecology and Obstetrics, ESIC-PGIMSR and ESIC Hospital, Joka, Kolkata were evaluated and those patients who meet the inclusion criteria were enrolled in the study. The patients were selected randomly. The detail history was taken; relevant clinical examination and all routine investigations were performed. A written consent was taken from every patient after full explanation of procedure. Age matched suitable normoglycemic or control was also selected in the study. Every patient was advised for at least 12 to 14 hours overnight fasting and 5 ml venous blood sample was collected in a disposable syringe on next morning for the estimation of blood glucose and glycated hemoglobin. Serum was separated and used for measurement of insulin, C-peptide, testosterone, FSH and LH levels. Semen samples were collected by masturbation from each diabetic patient of each group (regular and irregular treatment group) in a clean specimen container after sexual abstinence for 3 to 5 days followed by liquifaction at 37°C for the assessment of sperm count, sperm motility, viability, hypo-osmotic swelling test, nuclear chromatin decodensation, acrosomal status and seminal fructose level. Another part of the semen samples were centrifuged at 3000 × **g** for 10 min to obtain the seminal plasma and sperm pellet. The separated sperm pellet was stored at –80°C until further analysis for the detection of different antioxidative enzymes like catalase, peroxidase, superoxide dismutase as well as quantification of free radicals end products like conjugated diene (CD) and thiobarbituric acid reactive substances (TBARS). Age and nutritional status matched control were also collected from the society.

Experimental groups

Group-I (Control group): This group was consisted of healthy fertile normoglycemic individuals. All the samples belong to the age group of 25 to 40 years as well as same nutritional and same socioeconomic status. The same control group was used for all the three duration dependent study mode i.e. Group IV, Group VII.

Group II (Infertile Diabetic patients for 4 to 5 years under regular treatment mode): This group was composed of 150 male who suffering from diabetes and remaining in regular treatment mode for 4 to 5 years.

Group III (Infertile Diabetic patients for 4 to 5 years under irregular treatment mode): This group was consisted of 150 male diabetic patients under irregular treatment mode for 4 to 5 years.

Group IV (Control group): Same as group I

Group V (Infertile Diabetic patients for 6 to 10 years under regular treatment mode): This group was composed of 150 diabetic patients under regular treatment mode for 6 to 10 years.

Group VI (Infertile Diabetic patients for 6 to 10 years under irregular treatment mode): This group was consisted with 150 male diabetic patients for 6 to 10 years under irregular treatment.

Group VII (Control group): Same as group I

Group VIII (Infertile Diabetic patients under 11 to 15 years regular treatment mode): This group covered 150

male diabetic patients who were under regular treatment mode for 11 to 15 years.

Group IX (Infertile Diabetic patients under for 11 to 15 years irregular treatment mode): This group was composed of 150 male diabetic patients under irregular treatment mode for 11 to 15 years.

Estimation of fasting blood glucose and glycated hemoglobin level

Fasting blood glucose and glycated hemoglobin levels were quantified using the supplied kit (Crest Biosystems, Verna, India) following the supplied protocol as per standard guideline.

Sperm count, motility and viability test

Sperm count, motility and viability test were performed according to standard protocol (WHO, 1999).

Hypo-Osmotic Swelling (HOS) test

Sperm's membrane integrity was assessed by hypo-osmotic swelling (HOS) test. In brief, assay was performed by incubating 50 µl sperm suspension with 1 ml hypo-osmotic solution. Two hundred sperm were evaluated, and percentage of live sperm with coiled tail was calculated (Jeyendran *et al*., 1992).

Nuclear Chromatin De-condensation (NCD) test

Spermatozoa from seminal plasma were obtained by centrifugation at 400×g for 15 min and resuspended in borate buffer (0.05 M) containing dithiothreitol (2 mmol). They were then incubated for 30 min at room temperature.

Then, 0.1 mL of sodium dodecyl sulfate (0.1%) in sodium borate buffer was added, mixed gently and incubated for 2 min at room temperature. Sperms were fixed with 2.5% glutaraldehyde. An aliquot of the mixture was stained with 0.8% Rose Bengal and examined under a phase-contrast microscope (Rodriguez *et al*., 1985).

Acrosomal status assessment

The acrosome status was performed (Gopalkrishnan, 1995). A gelatin coat was prepared on a clean glass slide. Smear of the semen was prepared on the gelatin slide. The slide was incubated at 37°C for 2 h. Acrosomal enzymes dissolved the gelatin and created holes. Spermatozoa with holes were counted against those without holes, and the percentage was noted.

Estimation of serum insulin, C-peptide, testosterone, FSH and LH

Serum levels of insulin, C-peptide, testosterone, FSH and LH were measured by ELISA method using Ranbaxy Kit. Optical density was measured using ELISA reader (E.Merk – Mios-Mini) (Srivastava 2000).

Biochemical assay of catalase and peroxidase activities

The activities of catalase and peroxidase of sperm pellet were performed biochemically following standard protocol (Chatterjee *et al*., 2009). For evaluation of catalase and peroxidase activities in sperm pellet, the pellet was collected and homogenized in 0.5 M Tris-HCl buffer solution (pH 7.0). Homogenates were centrifuged at 10000×g at 4 ºC for 10 min and supernatant was used for the estimation of catalase and peroxidase activities.

Estimation of thiobarbituric acid reactive substance (TBARS) and conjugated diene (CD)

For quantification of end products of lipid peroxidation, that is, conjugated diene (CD) and thiobarbituric acid-reactive substance (TBARS), the sample tissue was homogenized separately at the tissue concentration of 50 mg/ml in 0.1 M of ice-cold phosphate buffer (pH 7.4), and the homogenates were centrifuged at 10000×g at 4°C for 5 min separately. Each supernatant was prepared for the spectrophotometrical estimation of CD and TBARS following the standard laboratory method (Ohkawa *et al*., 1979; Slater, 1984).

Estimation of seminal fructose level

The seminal plasma fructose level was measured in sample according to the standard method (Lu *et al*., 2007).The seminal plasma was deproteinized by adding 50 µl of zinc sulphate and 50 µl of sodium hydroxide to make a total dilution of seminal plasma 1:16, followed by centrifugation at 5000×g for 15 min. Clear supernatant was used in the volume of 200 µl for the analysis. The optical density of standard and sample were measured against blank at 470 nm. The concentration of fructose was obtained by plotting the value in standard curve and the value was expressed in unit of µM/ejaculate.

Statistical analysis

Data were expressed in mean ± SEM. For statistical analysis of data, Analysis of Variance (ANOVA) followed by multiple comparison two tail t-test was employed (Sokal and Rohle, 1997) and $p<0.05$ was considered significant.

RESULTS

Fasting blood glucose and glytated haemoglobin levels

Fasting blood glucose and glytated haemoglobin levels were significantly increased in diabetic patients under irregular treatment in respect to the diabetic patients under regular treatment as well as control. Significant increase in the levels of fasting blood glucose and glytated haemoglobin were noted along with the elevation in the duration of the irregular treatment to the diabetic patients in respect to 4 to 5 years diabetic patients under irregular treatment. However, the levels of the said parameters were significantly increased in diabetic patient under regular treatment in respect to control but the levels of these parameters were not significantly increased further along with the extension of the duration of the treatment in respect to 4 to 5 years duration of the treatment (Fig. 1).

Table 1. Duration dependent effect of regular and irregular treatment mode of diabetic patients on serum insulin, c-peptide, testosterone, LH and FSH levels. Data were expressed as Mean ± SEM (n=150) ANOVA followed by multiple comparision two tail t-test. Coloum with different superscripts (a, b, c, d, e) differ from each other significantly, p<0.05

Duration	Group	Serum Insulin (mIU/ml)	Serum C-peptide (ng/ml)	Serum testosterone (ng/ml)	Serum LH (mIU/ml)	Serum FSH (mIU/ml)
4-5 years	Control (Group I)	13.91 ±0.89^a	1.28 ±0.087^a	7.24 ±0.22^a	9.27 ±0.27^a	8.25 ±0.12^a
	Diabetic with regular treatment (Group II)	22.65 ±0.92^b	1.56 ±0.082^b	5.92± 0.18^b	6.72 ±0.21^b	6.12 ±0.15^b
	Diabetic with irregular treatment (Group III)	16.87 ±0.86^c	1.28 ±0.079^c	4.19 ±0.26^c	5.25 ±0.18^c	4.87 ±0.14^c
6-10 years	Control (Group IV)	14.21± 0.82^a	1.16 ±0.087^a	8.54 ±0.28^a	9.74 ±0.26^a	7.64 ±0.16^a
	Diabetic with regular treatment (Group V)	21.87 ±0.88^b	1.45 ±0.076^b	5.87 ±0.19^b	6.52 ±0.25^b	6.75 ±0.11^b
	Diabetic with irregular treatment (Group VI)	11.76 ±0.82^d	1.18± 0.079^d	3.18 ±0.20^d	4.32 ±0.24^d	3.87 ±0.15^c
11-15 years	Control (Group VII)	12.85 ±0.79^a	1.32 ±0.087^a	7.32 ±0.24^a	8.94 ±0.27^a	8.68 ±0.14^a
	Diabetic with regular treatment (Group VIII)	20.76 ±1.02^b	1.42 ±0.086^b	5.22± 0.24^b	6.22 ±0.19^b	6.58 ±0.12^b
	Diabetic with irregular treatment (Group IX)	11.62 ±0.84^e	1.06 ±0.080^e	2.61 ±0.18^e	3.25 ±0.18^e	2.81 ±0.14^e

Table 2 Changes in sparmatological parameters (sperm count, motility, viability, HOS, NCD, acrosomal status) in diabetic patients under different durations of regular treatment or irregular treatment mode. Data were expressed as Mean ± SEM (n=150) ANOVA followed by multiple comparision two tail t-test. Coloum with different superscripts (a, b, c, d, e) differ from each other significantly, p<0.05

Duration	Group	Sperm count (million/ml)	Sperm motility (%)	Sperm viability (%)	HOS of sperm (%)	NCD of sperm (%)	Acrosomal status (%)
4-5 years	Control (Group I)	133.24 ±2.81^a	88.24 ±3.81^a	92.32 ± 3.35^a	82.14 ±3.12^a	80.24 ±3.17^a	84.56 ±4.92^a
	Diabetic with regular treatment (Group II)	103.25 ±3.12^b	72.32±4.72^b	68.52 ± 2.54^b	71.43± 2.24^b	68.95 ±3.25^b	68.42 ±4.75^b
	Diabetic with irregular treatment (Group III)	74.23 ±4.46^c	48.24 ±4.45^c	41.26 ± 3.10^c	42.35 ±3.18^c	44.56 ±4.16^c	48.75 ±4.84^c
6-10 years	Control (Group IV)	128.22± 2.72^a	86.45 ±3.71^a	91.42 ± 3.15^a	80.18 ±3.46^a	84.76 ±3.24^a	82.16 ±4.52^a
	Diabetic with regular treatment (Group V)	98.27 ±3.41^b	68.24 ±5.79^b	64.25 ±3.42^b	68.97 ±3.21^b	65.54 ±3.75^b	65.45 ±4.61^b
	Diabetic with irregular treatment (Group VI)	56.61 ±4.34^d	34.21± 4.68^d	32.26 ± 3.41^d	34.25 ±4.25^d	32.47 ±4.04^d	38.94 ±4.85^d
11-15 years	Control(Group VII)	138.18 ±2.43^a	84.46 ±3.22^a	90.42 ± 3.42^a	84.22 ±3.65^a	81.56 ±3.47^a	86.46 ±4.32^a
	Diabetic with regular treatment (Group VIII)	108.36 ±4.02^b	68.95 ±4.84^b	65.32 ± 2.47^b	70.24± 4.42^b	64.56 ±3.12^b	62.46 ±4.82^b
	Diabetic with irregular treatment (Group IX)	42.62 ±3.16^e	18.96 ±3.90^e	28.14 ± 2.08^e	21.54 ±3.17^e	21.32 ±3.16^e	18.26 ±4.94^e

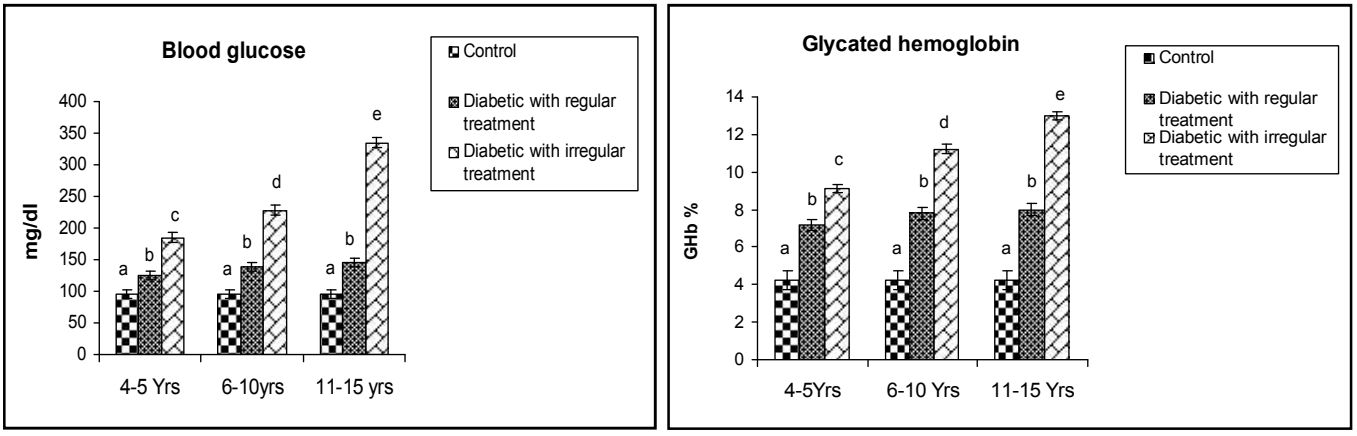

Figure 1. Variation in the levels of fasting blood glucose and glytated haemoglobin among the diabetic patients under regular and irregular treatment mode for different durations. Bars were expressed as Mean ± SEM (n = 150). ANOVA followed by multiple comparison two-tail t-test. Bars with different superscripts (a, b, c, d, e) differ from each other significantly, p< 0.05

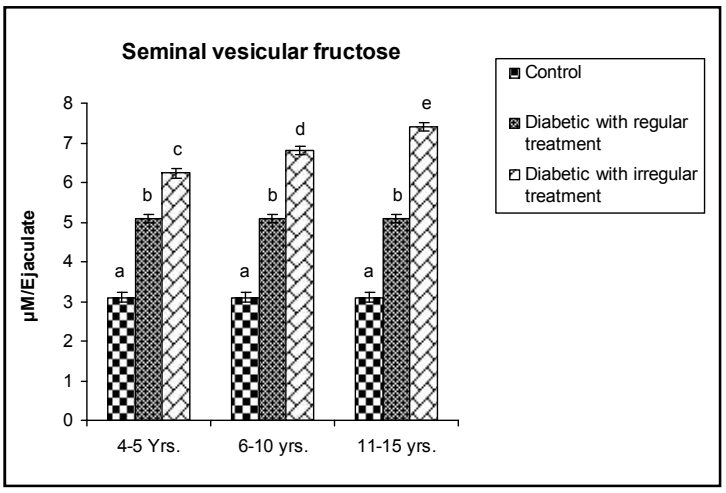

Figure 2. Changes in the seminal vesicular fructose levels in diabetic patients under regular or irregular treatment mode for different durations. Bars were expressed as Mean ± SEM (n = 150). ANOVA followed by multiple comparison two-tail t-test. Bars with different superscripts (a, b, c, d, e) differ from each other significantly, p< 0.05

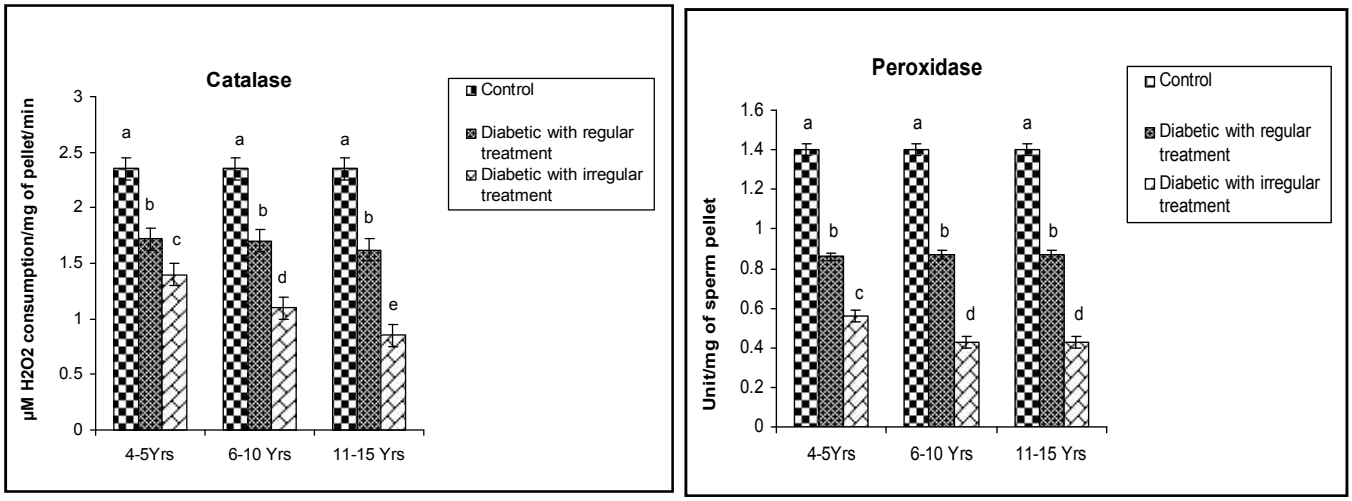

Figure 3. Alteration in the activities of catalase and peroxidase in sperm pellet among the diabetic patients of different duration dependent treatment under regular and irregular treatment mode. Bars were expressed as Mean ± SEM (n = 150). ANOVA followed by multiple comparison two-tail t-test. Bars with different superscripts (a, b, c, d, e) differ from each other significantly, p< 0.05

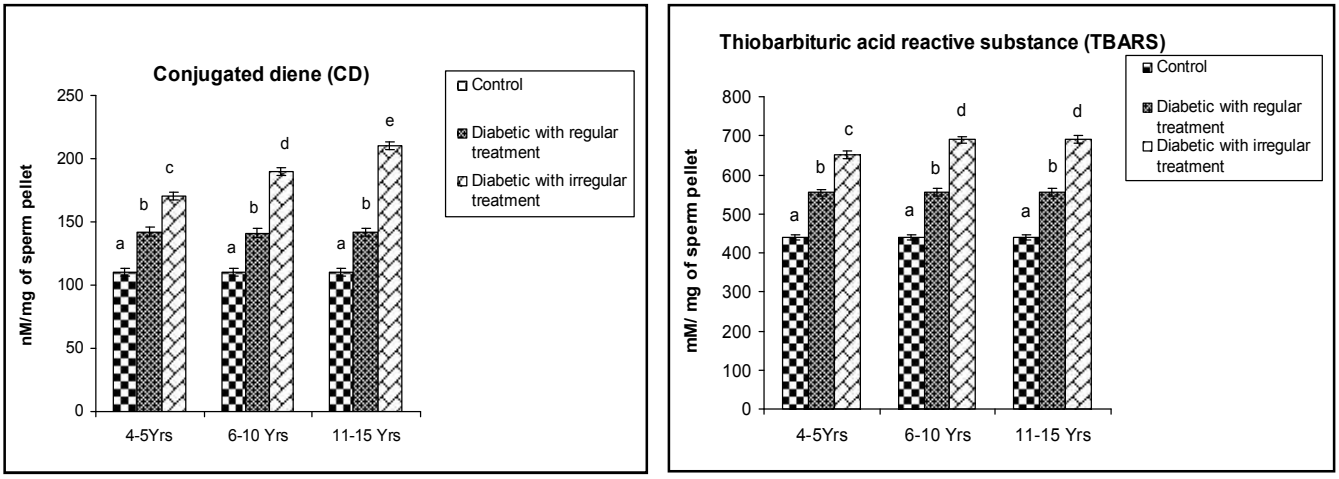

Figure 4. Change in the levels of CD and TBARS in sperm pellet among different treatment duration groups in the diabetic patients under regular and irregular treatment schedule. Bars were expressed as Mean ± SEM (n = 150). ANOVA followed by multiple comparison two-tail t-test. Bars with different superscripts (a, b, c, d, e) differ from each other significantly, p< 0.05

Serum C-Peptide and insulin level

Serum C-peptide and insulin levels were increased significantly in diabetic patients under regular treatment in respect to control. These levels were not increased further along with extension of the duration of the regular treatment beyond 4 to 5 years up to 11 to 15 years. In diabetic patients under irregular treatment, levels of the said sensors were decreased significantly with respect to diabetic patients under regular treatment. In contrast, the levels of above sensors were decreased significantly along with elevation in the duration of irregular treatment in respect to 4 to 5 years of treatment (Table 1).

Levels of serum testosterone, LH and FSH

Serum testosterone, LH and FSH levels were significantly decreased in diabetic patients under irregular treatment in respect to the diabetic patients under regular treatment in different duration dependent group. When the duration of irregular treatment has been increased from 4 to 5 years to 11 to 15 years duration, the levels of the said parameters were decreased significantly in respect to 4 to 5 years irregular treatment mode. However, the significant decreased in the levels of said parameters were recorded in diabetic patients under regular treatment in respect to control though the levels were not further decreased significantly beyond 4 to 5 yrs of regular treatment duration.(Table 1).

Spermatogenic profile

Levels of spermatological profile i.e. sperm count, motility, viability, hypo-osmotic swelling test, nuclear chromatin decondation test, and acrosomal test were significantly decreased in diabetic patient under irregular treatment and diabetic patients under regular treatment with respect to the control. In case of regular treatment, the levels of said sensors were not significantly decreased further along with the extension of the treatment duration beyond 4 to 5 years of regular treatment. In contrast the levels of above sensors were decreased significantly along with elevation in the duration of irregular treatment in respect to 4 to 5 years of treatment (Table 2).

Seminal fructose level

The level of seminal fructose was significantly increased in diabetic patients under irregular treatment or diabetic patients under regular treatment in respect to the corresponding control. The level of seminal fructose was increased significantly in diabetic patient under irregular treatment mode for 6 to 10 years or beyond that in comparison with 4 to 5 years treatment group in duration dependent manner. The level of this sensor was not significantly altered along with elevation in the duration of regular treatment in respect to 4 to 5 years of treatment (Fig 2).

Catalase and peroxidase activity in sperm pellets

Activities of catalase and peroxidase in sperm pellet were significantly reduced in diabetic patients under irregular or regular treatment in respect to the corresponding control. The activities of the said enzymes were further decreased along with elevation in the duration in irregular treatment beyond 4 to 5 years of treatment schedule and in some cases this diminution was continued up to the 11 to 15 years of irregular treatment. Sperm pellet catalase and peroxidase activities were not significantly decreased further along with the extension of the treatment duration in respect to 4 to 5 years of diabetic patients under regular treatment mode (Fig 3).

Level of TBARS and CD

Levels of TBARS and CD of sperm pellet were increased significantly in graded manner in duration dependent fashion in diabetic patients who are under irregular treatment in respect to diabetic patients under regular treatment as well as in comparison with the control. Though, the levels of these parameters were significantly increased in diabetic patients under regular treatment in respect to the control but these levels were not significantly changed along with the elevation in the treatment when compared with 4 to 5 years of treatment schedule (Fig 4).

DISCUSSION

In the present study elevation in fasting blood glucose level in diabetic patients was noted and the excess glucose present in blood reacts non enzymatically with haemoglobin which is an oxidative reaction and form glytated haemoglobin (Klein, 1995, De *et al*., 2010). The elevation in insulin which has been noted in regular treatment mode may be due to regular basis of intramuscular injection of insulin or other drugs those stimulate β-cells for insulin secretion that also results elevation in C-peptide in serum. Instead of that blood glucose and glytated haemoglobin levels were not resettled to the control which may be due to desensitization of insulin receptor to insulin. Deviation in the levels of spermiological parameters i.e. sperm count, motility, viability, hypo-osmotic swelling test, nuclear chromatin decondensation test, which were decreased in diabetics patients under regular and irregular treatment may be due to inhibition in spermatogenesis or may be due to oxidative free radicals deposition in sperm and diminution in the activities of antioxidant enzymes in sperm pellet. The oxidative stress induction in sperm in diabetic state may be due to high level of uncoupler protein synthesis in sperm or may be due to hypoxic state condition in testis as diabetes results glytated haemoglobin formation that interferes oxygen delivery at target tissue (Stabler *et al*., 2010). This has been proved here by the study of decreased catalase and peroxidase activities in sperm pell*et al*ong with the increased levels of end products of free radicals like CD and TBARS in diabetic patients under regular treatment and irregular treatment. In regular treatment group though the levels of oxidative stress has been corrected partially after 4 to 5 yrs treatment but there was no further significant recovery in this concern. This may be due to the capacity in the recovery of oxidative stress which has specific limits and beyond that limits extension of treatment is unable for resettled the sensor to the control level. In contrast irregular treatment is more dangerous because the oxidative stress injury markers are further elevated beyond the duration of 4 to 5 yrs. This may be

explained that intermittened irregular treatment may impose chemical stress on the said organ (Aitken *et al*., 1992). Levels of seminal plasma fructose which is the main energy source for sperm viability and motility has been increased in diabetes patient under regular or irregular treatment in respect to control and this may be due to the diminution of the sperm count that may interferes in fructose utilization (Naha and Manna, 2007). Serum testosterone LH and FSH all were affected drastically in diabetic patients under irregular treatment which may affect the spermatological sensors more drastically than the regular treatment. This diminution in spermatological sensors and gonadotropin in irregular treatment may be due to incomplete recovery process that affects the cellular physiology more drastically.

Conclusion

From the result it may be concluded that regular treatment is maximum beneficial for recovery of testicular hypofunction in diabetes patient in respect to irregular treatment. Moreover this irregular treatment mode is more harmful rather than recovery. This message may be dissipated to the diabetic community for the generation of health awareness in this concern.

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