Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 9, Issue, 04, pp.49289-49295, April, 2017

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

APOPTOTIC AND ANTIOXIDATIVE STATUS OF SPERM IN DIABETIC PATIENTS OF 25-40 YEARS AGE GROUP UNDER DURATION DEPENDENT REGULAR AND IRREGULAR MODE OF TREATMENT: A COMPARATIVE APPROACH OF PROTEOMIC AND GENOMIC STUDY

[1, 2]Sumanta Bera, [1]Abhinandan Ghosh, [2]Bhabani Prasad Pakhira, [2]Joydeb Roychowdhury and [1,*]Debidas Ghosh

[1]Nutrigenomics and Molecular Medicine Research Laboratory, Bio-Medical Laboratory Science and Management, Vidyasagar University, Midnapore-721102, West Bengal, India
[2]Department of Pathology and Department of Gynecology and Obstetrics, ESIC-PGIMSR, ESIC Hospital, Joka, Kolkata-700104

ARTICLE INFO

Article History:
Received 17th January, 2017
Received in revised form
14th February, 2017
Accepted 09th March, 2017
Published online 30th April, 2017

Key words:

Diabetes,
Spermiological Hypofunction,
Oxidative stress,
Regular Treatment Mode,
Irregular Treatment Mode.

ABSTRACT

Objective: The present study was undertaken to search out the status of antioxidant enzymes, apoptotic markers of sperm pellet and their molecular mechanism study in the diabetic patients under regular and irregular mode of different duration dependent treatment protocol within 25-40 years age group.

Methods: Blood glycemic sensors, seminal catalase, superoxide dismutase (SOD), Bax, Bcl-2 protein, transcriptions of catalase, SOD, Bax and Bcl-2 genes in sperm were evaluated.

Results: Levels of blood glycemic sensors, activities of antioxidative enzymes like catalase and superoxide dismutase and translation value of apoptatic markers like Bax protein, Bcl-2 protein, and transcriptions of catalse, SOD, Bax and Bcl-2 genes in sperm was corrected significantly after treatment of diabetic patients under regular mode for 4 to 5 years though not up to the control level. When the duration of the said treatment was extended beyond 4-5 years up to 15 years there are no significant recovery further in this line. In contrast, the levels of these sensors were shifted towards the pathological direction further along with the extension of the duration of the irregular treatment more in comparison to the irregular pattern treatment for 4-5 year of the diabetic patients..

Conclusion: So, it may conclude that regular mode of treatment is more effective for correction of spermiological sensors in diabetic patients in respect to irregular mode of treatment. Moreover, irregular mode of treatment with prolog duration may exert more adverse effect on spermiological sensors in diabetic patients.

Citation: Sumanta Bera, Abhinandan Ghosh, Bhabani Prasad Pakhira, Joydeb Roychowdhury and Debidas Ghosh. 2017. "Apoptotic and antioxidative status of sperm in diabetic patients of 25-40 years age group under duration dependent regular and irregular mode of treatment: A comparative approach of proteomic and genomic study", *International Journal of Current Research*, 9, (04), 49289-49295.

INTRODUCTION

Chronic diabetes mellitus (DM) is considered as the major metabolic disorder with stress induced health hazard in modern public health system. Factors such as obesity, population expansion and ageing are thought to be largely accountable (Andy and Alberti, 2009). Men suffering from DM prior to and during their vital reproductive age results more complication in their sexual events (Agbaje *et al*., 2007; Shrilatha, 2007).

****Corresponding author: Debidas Ghosh,***
Nutrigenomics and Molecular Medicine Research Laboratory, Bio-Medical Laboratory Science and Management, Vidyasagar University, Midnapore-721102, West Bengal, India.

Different kinds of structurally and physiologically male reproductive dysfunctions were studied by several workers in cases of diabetes mellitus Ricci *et al*., 2009; O'Neill *et al*., 2010). Diabetes mellitus may exerts undesirable effects on male gametogenic activities at multiple levels including its deleterious effects on hormonal regulation of spermatogenesis and/or by interfering penile erection and semen ejaculation. Diabetes is associated with reduced semen volume and decreased sperm vitality and motility without any remarkable alteration in seminal viscosity Andy and Alberti, 2009; Ricci *et al*., 2009). There are some confirmations indicating higher rates of infertility in diabetic men and poor reproductive outcomes in comparison with healthy men Amaral *et al*., 2008). Sperm plasma membrane integrity is affected by oxidative stress along with integrity of sperm nuclear DNA (Aitken, 1999). Oxidative stress induced single and double DNA strand breakage is

highly suspicious in diabetes (Twigg *et al*., 1998). Spermatozoa of infertile men showed DNA fragmentation which is induced by high levels of ROS (Kodama *et al* 1996; Sun *et al*., 1997). Though, there are several works focusing diabetes induced DNA damage in sperm in model animals but very few studies have yet been conducted in human model on apoptotic markers of sperm cells and its mechanism in a duration dependent manner. The rationale of this study is to find out the status of antioxidative enzymes and the apoptatic markers in relation with semen quality of diabetic individual (25-40 yrs age group) under regular and irregular mode of therapeutic treatment for different duration.

MATERIALS AND METHODS

Patient selection

Patients who are diabetic under regular treatment or diabetic 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 below the age of 40 years were excluded from the study. Male diabetic as well as hypogonadic patients were included and those patients who meet the inclusion criteria were enrolled in the study. Selection of the patients were selected randomly. The clinical history in detail, relevant medical examination and relevant routine investigations were covered. patients written consent was taken from each and every patient after explanation of the purpose of the study. The work was approved by the Institutional Ethics Committee of ESI-PGIMSR and ESIC Hospital, Joka, Kolkata. Age matched suitable normoglycemic or control was also selected in the study.

Experimental Design

The current study was performed in the Department of Pathology and Department of Gynecology and Obstetrics, ESIC-PGIMSR, ESIC Hospital, Joka, Kolkata, in collaboration with the Department of Bio-Medical Laboratory Science and Management, Vidyasagar University, Midnapore, West Bengal. The present study consists of 450 cases of chronic diabetic under regular treatment and 450 cases of chronic diabetic under irregular treatment with infertility complication in the age of 25 to 40 years. The results were compared with the values of 150 apparently healthy non-diabetic individuals which were in the same age group. Routine spermiological analysis, glycemic study were conducted of all the patients but biochemical analysis, proteonomic and genomic analysis were followed to 15 patients of each subgroups for constraint of funds as chemicals are highly expensive. Such samples were selected randomly from the patient community fulfilling all the criteria. Subjects were the patients who attended for checkups and treatment in the said departments.

Diabetic patients were grouped first into three groups on the basis of duration of diabetes i.e 4-5 years, 6-10 years and 11-15 years. Each group was then subdivided into two subgroups from the viewpoint of regular and irregular mode of treatment. Control group was included in all duration dependent group where individual have normal blood glucose level and male gonadal activity with same age. Each of the patients was advised for 10 to 12 hours overnight fasting at least and 5 ml blood sample was collected in a disposable syringe on next morning for the estimation of blood glucose and glycated hemoglobin.

All the diabetic patients was requested to collect semen by masturbation (regular and irregular treatment group) in a sterile wide mouth semen collection container after sexual abstinence for 3 to 5 days. After liquifation of the semen sample at 37^{o}C followed by routine examination of the semen samples were centrifuged at 3000 × **g** for 10 min for collection sperm pellet and plasma fraction. The sperm pellet was preserved at –80°C till future analysis in connection with the proteonomic and genomic studies of catalase, SOD, Bax protein and Bcl-2 protein in sperm pellet. Age and nutritional status matched control were also collected from the same socioeconomic status.

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 respective control groups were used for all three duration dependent study mode i.e. Group I, Group IV and Group VII. Group II (Diabetic patients for 4 to 5 years under regular treatment mode): This group covered 150 male patients who were suffering from diabetes and remaining in regular treatment mode for 4 to 5 years. Out of 150 patients, infertile individuals were fifty five. Group III (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, infertile individuals were hundred eight.

Group IV (Control group): Same as group I

Group V (Diabetic patients for 6 to 10 years under regular treatment mode): There were 150 patients under this group. Patients were under regular treatment mode for 6 to 10 years. There were 35 infertile diabetic patients in this group out of one hundred fifty.

Group VI (Diabetic patients for 6 to 10 years under irregular treatment mode): This group covered 150 male diabetic patients for 6 to 10 years under irregular treatment. Where, the infertile patients were seventy four.

Group VII (Control group): Same as group I

Group VIII (Diabetic patients under for 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. Out of 150 patients, infertile diabetic patient was twenty eight.

Group IX (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. The numbers of infertile patients were sixty five.

Semen processing

Semen was collected from each patient as per the method mentioned earlier.

Estimation of blood glucose level

Blood glucose level of fasting and post-prandial state were measured with the help of Glucose Kit of E.Merk (GOD-POD) (Trinder *et al*., 1969).

Estimation of glycated hemoglobin

By HPLC with D-10, Bio-Rad instrument, glycated hemoglobin (HbA1C) was estimated (Goldstein *et al*., 1986).

Catalase activity assessment

The catalase activity of sperm pellets were assessed according to standard protocol (Beers and Sizer, 1952). At first 0.5 ml of 0.00035 mmole H_2O_2 mixed with distilled water(2.5 ml) and absorbance was noted at 240 nm as initial reading. Processed sample(40 µl) were added to the cuvette and the consecutive six readings were noted at interval of 30 second.

Assessment of superoxide dismutase (SOD) activity

The activity of SOD of the sperm pellet was measured in terms of percentage of inhibition in the pyrogallol auto oxidation by SOD (Marklund and Marklund, 1974). In a spectrophotometric cuvette, Tris buffer (2.04 ml), (50 mmole, pH-8.2), 20 µl of sample and 20 µl of pyrogallol were taken. Absorbance was noted for 3 min period in spectrophotometer at 420 nm. The enzyme activity that inhibits the auto-oxidation of pyrogallol by 50 % known as unit of SOD.

RNA isolation and complementary DNA synthesis

Total RNA was extracted using kit (Roche Diagnostic, Mannheiem, Germany), and cDNA was synthesized using 'Transcriptor First Strand cDNA Synthesis Kit' (Roche Diagnostic) (Ghosh *et al*., 2016).

Realtime polymerase chain reaction or Quantitative reverse transcription polymerase chain reaction (qRT-PCR) analysis

Assessment of transcription of the following genes of sperm pellet, i.e catalase, SOD, Bax, Bcl-2 Light Cycler 480 II (Roche Diagnostic) (Ghosh *et al*., 2016).

Western blot analysis

Analysis of Bax and Bcl-2 protein was carried out through western blot(NP-40 of 1%, sodium deoxycholate of 0.5%, SDS of 1 % in PBS with protease inhibitor) (Maheshwari *et al*., 2009). At first frozen sperm pellet were placed in 3 ml ice-cold RIPA buffer per gram of sperm pellet and the sperm pellet was homogenized and incubated in ice for half an hour. Centrifugation of the lysates were performed at 10,000 g for 10 minutes at 4°C. Quantification of protein was done biochemically (Bradford assay) (Bradford, 1976). In a mini-protean cell,western blot analysis was performed by resolving 30–50 mg protein on a 12.5% SDS polyacrylamide gel at 100V (Bio-Rad). Nitrocellulose membranes was used to transfer protein in transfer buffer (Tris base 25 mM, glycine 190 mM, methanol 20 %) at 100V in the cold chamber for one hour. Blocking of the membranes were performed by using PBS having 0.05% Tween-20 and 5% nonfat milk. The preparation was incubated at 4°C for overnight in presence of primary antibody (1:500 Bax, 1:400 Bcl-2). Membranes were washed in PBS with Tween 80 **(**PBST) and incubated further incubated with secondary antibody(Horseradish peroxidase-conjugated goat anti-rabbit (Santa Cruz Biotechnology) at 1:2000 dilutions. As an internal control β -actin was used as reference. Bands were visualized by using substrate (tetramethyl benzidine/ hydrogen peroxide (TMB/ H_2O_2) Immunospecfic densitometric was performed for quantification of immunospecific bands..

Statistical analysis

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

RESULTS

Blood glucose

Patients suffering from diabetes for last 11-15 years under irregular treatment showed significantly high blood glucose level in respect to the patients under same treatment mode for 4-5 years and 6-10 years. Diabetic patients under regular treatment mode did not show any significant alteration among 4-5 years, 6-10 years and 11-15 years of diabetes suffering. Blood glucose level of the diabetic patients under irregular treatment mode significantly differed from regular treatment mode as well as control (Fig. 1).

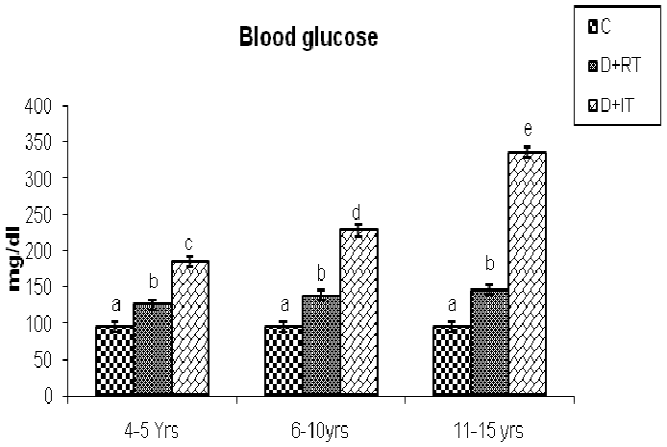

Figure 1. Variation in the level of blood glucose among the diabetic patient under regular and irregular treatment depending on advancement of duration. Bars were expressed as Mean ± SEM (n = 150). ANOVA followed by multiple comparison two-tail t-test. Bars with different superscripts (a, b, c, d, e) differ from each other significantly, p< 0.05

Level of glycated hemoglobin (HbA1C)

Diabetic patients suffering for 11-15 years under irregular mode of treatment showed significantly higher ($p<0.05$) level of glycated haemoglobin in compare to diabetic patients under irregular treatment of duration of 4-5 years and 6-10 years. Where as in case of diabetic patients under regular treatment of 4-5 years, 6-10 years and 11-15 years the level of glycated haemoglobin showed significant recovery ($p<0.05$) but not resettled to the control. Glycated haemoglobin level of the diabetic patients under irregular treatment mode significantly differed from regular treatment mode as well as control (Fig. 2).

Catalase activity in sperm pellets

Sperm pellet catalase activity in diabetic patients were significantly recovered towards the control after regular

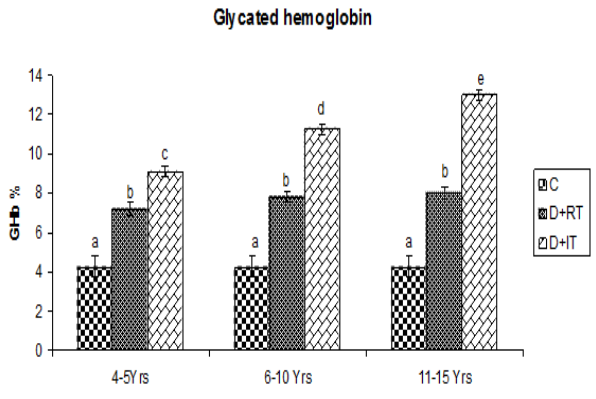

Figure 2. Deviation in the level of glycated haemoglobin in blood among the diabetic patient under regular and irregular treatment depending on advancement of duration. Bars were expressed as Mean ± SEM (n = 150). ANOVA followed by multiple comparison two-tail t-test. Bars with different superscripts (a, b, c, d, e) differ from each other significantly, p< 0.05

treatment of 4-5 years but there is no further recovery inspite of The significant recovery (p<0.05) in the activity of catalase was noted towards control in sperm pellet of diabetic patients under regular mode of treatment of 4-5 years, 6-10 years and 11-15 years but not resettled to the control. Diabetic patients suffering for 11-15 years under irregular mode of treatment showed significantly lower (p<0.05) level of catalase activity in respect to diabetic patients under irregular treatment of duration of 4-5 years and 6-10 years. Catalase activity in sperm pellet was significantly (p<0.05) diminished in the diabetic patient under irregular treatment mode along with gradually increase of duration of suffering in diabetes. The catalase activity in sperm pellet of diabetic patients under irregular treatment mode significantly differed from regular treatment mode as well as control (Fig.3).

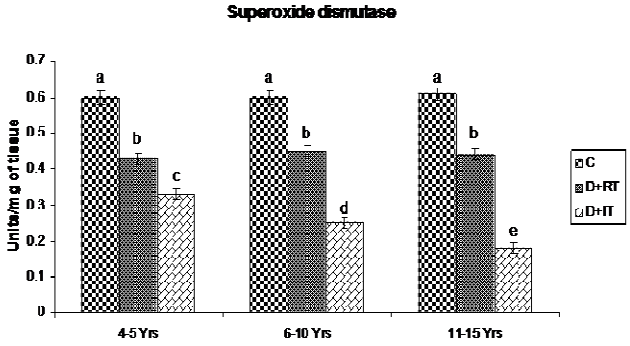

Figure 3. Alteration in the activity of catalase of sperm pellet among the diabetic patient under regular and irregular treatment depending on advancement of duration. Bars were expressed as Mean ± SEM (n = 150). ANOVA followed by multiple comparison two-tail t-test. Bars with different superscripts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

Activity of superoxide dismutase in sperm pellets

Patients suffering from diabetic for 11-15 years under irregular mode of treatment showed significantly lower (p<0.05) level of super oxide dismutase activity in respect to diabetic patients under irregular treatment of duration for 4-5 years and 6-10 years. The significant recovery (p<0.05) in the activity of superoxide dismutase was noted towards the control was noted in sperm pellet of diabetic patients under regular mode of treatment of 4-5 years, 6-10 years and 11-15 years but not resettled to the control. The activity of superoxide dismutase in sperm pellet of diabetic patients under irregular treatment mode significantly differed from regular treatment mode as well as control (Fig. 4).

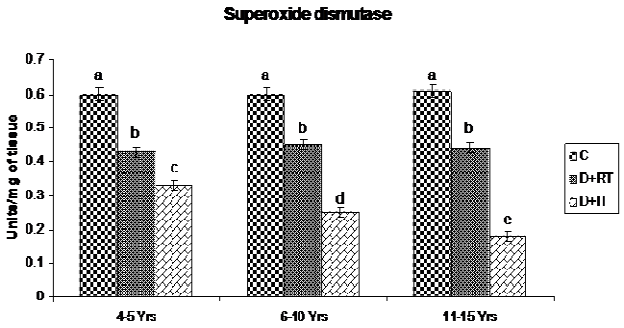

Figure 4. Divergence in the activity of superoxide dismutase in sperm pellet among the diabetic patient under regular and irregular treatment depending on advancement of duration. Bars were expressed as Mean ± SEM (n = 150). ANOVA followed by multiple comparison two-tail t-test. Bars with different superscripts (a, b, c, d, e) differ from each other significantly, p< 0.05

Western blot analysis of Bax protein from sperm pellet

Diabetic patients under irregular treatment mode showed higher expression of Bax protein in sperm pell*et al*ong with gradual increase in the duration of same treatment mode in respect to the diabetic patients under regular treatment and the control as well. The diabetic patients followed regular treatment showed maximum recovery after 4-5 years of treatment (Fig. 5).

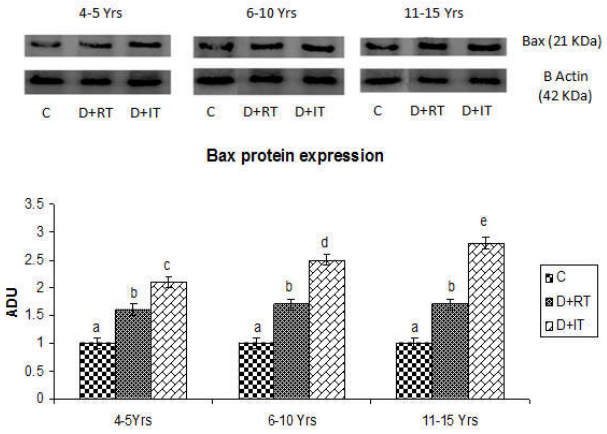

Figure: 5 Western blot analysis of Bax in sperm pellet of control individual, diabetic individuals under regular and irregular treatment followed by its densitometric analysis considering β-actin as reference. Each bar represents mean ± SEM (n=15). ANOVA followed by multiple comparisons two tail-t-test. Bar diagram with different superscripts (a,b,c,d,e) differ from each other significantly, p<0.05. [Lane 1: Control group (C); Lane 2: Diabetic patients under regular treatment (D+RT); Lane 3: Diabetic patients under irregular treatment (D + IT); A.D.U. – Arbitrary Densitometric Units]

Western blot analysis of Bcl-2 protein from sperm pellet

Bcl-2 protein expression in sperm pellet was significantly down regulated (p<0.05) in diabetic patients under irregular treatment group with respect to the diabetic patients under regular treatment group as well as control group. However, in diabetic patients under regular treatment, Bcl-2 protein expression was significantly downward in respect to the control group. When comparison made among different duration, no significance variation of Bcl-2 protein expression was noted in diabetic patients under regular treatment group. But in case of diabetic

patients under irregular treatment group showed gradual downward expression of Bcl-2 protein in between 4-5 years and 6-10 years of duration suffering in diabetes, but no significance deference was observed in between 6-10 years and 11-15 years diabetic suffering group (Fig. 6).

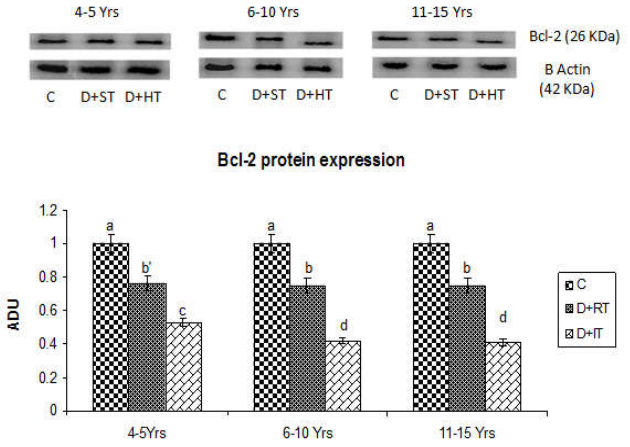

Figure 6. Densitometric analysis of Bcl-2 protein expression in sperm pellet of control individual, diabetic individuals under regular and irregular treatment considering β-actin as reference. Each bar represents mean ± SEM (n=15). ANOVA followed by multiple comparisons two tail-t-test. Bar diagram with different superscripts (a,b,c,d,e) differ from each other significantly, p<0.05. [Lane 1: Control group (C); Lane 2: Diabetic patients under regular treatment (D+RT); Lane 3: Diabetic patients under irregular treatment (D + IT); A.D.U. – Arbitrary Densitometric Units]

mRNA expression of Bax, Bcl-2, catalase and SOD genes by qRT-PCR study from sperm pellet

Transcript levels of Bax in sperm pellet was significantly up regulated whereas transcript level of Bcl-2 in the same tissue was significantly down regulated in diabetic patient under irregular treatment in respect to diabetic patient under regular treatment in duration dependent fashion. But in case of diabetic patients under regular treatment showed significant recovery in the expression pattern towards the control but not to the control level (Fig. 7-10)

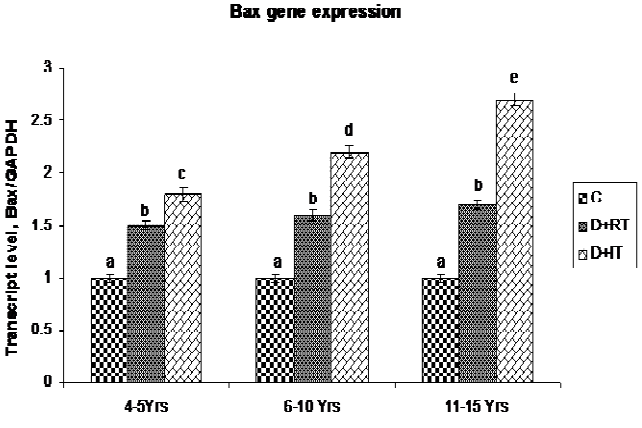

Figure 7: Changes in the expression of Bax gene among the diabetic patient under regular and irregular treatment depending on advancement of duration. Bars were expressed as Mean ± SEM (n = 15). ANOVA followed by multiple comparison two-tail t-test. Bars with different superscripts (a, b, c, d, e) differ from each other significantly, p< 0.05

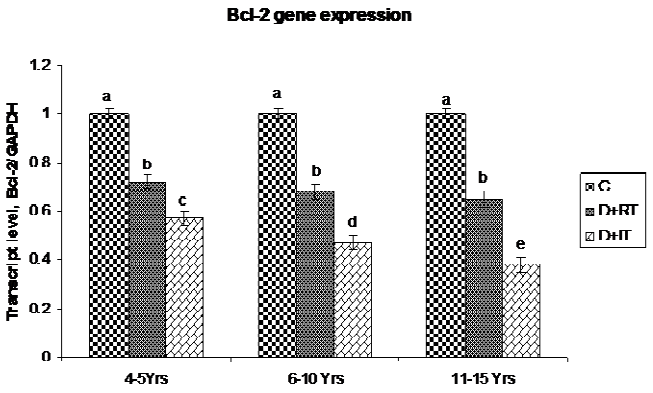

Figure 8. Deviation in the Bcl-2 gene expression among the diabetic patient under regular and irregular treatment depending on advancement of duration. Bars were expressed as Mean ± SEM (n = 15). ANOVA followed by multiple comparison two-tail t-test. Bars with different superscripts (a, b, c, d, e) differ from each other significantly, p< 0.05

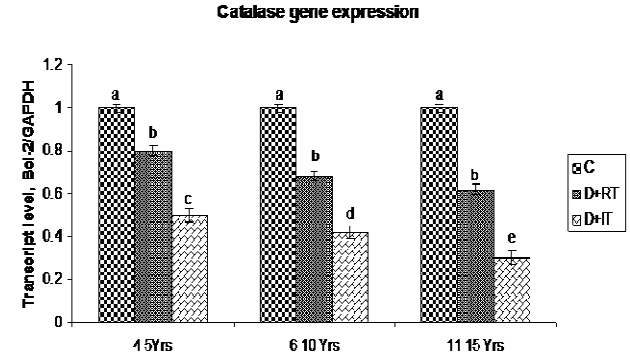

Figure 9. Variation in catalase gene expression among the diabetic patient under regular and irregular treatment depending on advancement of duration. Bars were expressed as Mean ± SEM (n = 15). ANOVA followed by multiple comparison two-tail t-test. Bars with different superscripts (a, b, c, d,e) differ from each other significantly, p< 0.05

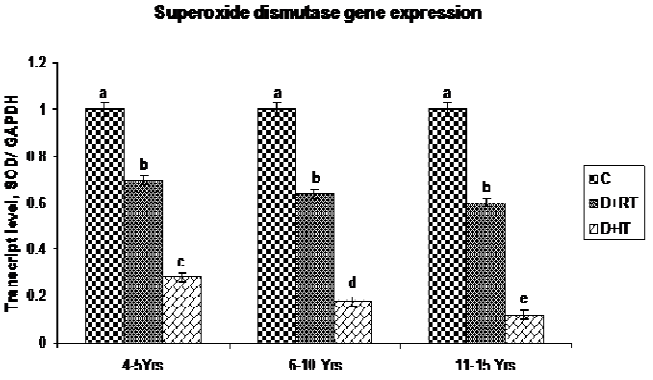

Figure 10. Alteration in the expression of superoxide dismutase gene among the diabetic patient under regular and irregular treatment depending on advancement of duration. Bars were expressed as Mean ± SEM (n = 15). ANOVA followed by multiple comparison two-tail t-test. Bars with different superscripts (a, b, c, d, e) differ from each other significantly, p< 0.05

DISCUSSION

In diabetic patients the excess glucose present in blood reacts non enzymatically with haemoglobin at the time of erythropoesis in the intermediate reticulocyte stage which is an oxidative reaction and form glycated haemoglobin (Klein,

1995). Similarly, elevation in the level of glycated hemoglobin in diabetic patients confirms the chronic diabetic state as glycated hemoglobin is the sensitive biomarker for confirmation of the diabetic condition (Mallick *et al*., 2010). Oxidative stress imposition in germ cell of diabetic patients in duration dependent fashion has developed at different duration which has been assessed here from the diminution in the activities of catalase and superoxide dismutase. Some workers also reported that reactive oxygen species (ROS) play a pivotal role in apoptosis of testicular germ cell in diabetic condition (Moustafa *et al*., 2004). Super oxide dismutase is considered as a first line of defense against oxygen toxicity and central regulator of ROS level by catalyzing the dismutation of super oxide radicals to H_2O and molecular O_2 (Bannister and Bannister, 1987). Expression pattern of antioxidant enzymes in sperm pellet has been considered here to find out whether diabetes induced oxidative stress imposition has any effect at genomic level beside its direct effect on catalyzing protein. In this present work it has been focused that regular treatment of diabetic patient may results significant recovery in the activities of the said enzymes under regular treatment for 4-5 years which was not further recovered after extension of treatment. In contrast, irregular treatment mode is more harmful than regular treatment mode. Elongation of such treatment resulted further detoriation of the sensors.

This may be due to withdrawal of drug as irregular mode of treatment may trigger such gene expression towards negative side by drug nutrient interaction. Germ cell apoptosis which is related to diabetes and the corrective measures by regular treatment remain unclear. On that background, to find out the molecular mechanism of diminution in spermatozoa in diabetes, the transcription of different apoptotic indicators were analyzed as the diminution in their transcription in spermatozoa closely related with apoptosis (Yan *et al*., 2000). Bax and Bcl-2 which were Pro-apoptotic marker and anti-apoptotic marker are also considered as oxidative stress induced apoptosis of the spermatozoa closely linked with each other (Maheshwari *et al*., 2009). Here, the gene expression study of Bax in sperm pellet resulted the elevation in the expression in diabetes patients, which was corrected towards the control in regular treatment group.

Similarly low level of expression of Bcl-2 in sperm pellet of diabetic patients was recovered by regular treatment. The result of present study was further strengthened here from the protein expression of Bax and Bcl-2 in sperm pellet by the densitometric analysis. Proteomic study is essential to unfold the post transcriptional modification if any through Western blot. It has been noted that regular treatment of diabetic patient can able to recover Bax and Bcl-2 protein in sperm pellet at significant level which was not possible by irregular mode of treatment of the patients. The translation of Bax and Bcl-2 in spermatozoa in relation to oxidative stress was further supported by our previous publication conducted on animal model Ghosh *et al*., 2016; Ray *et al*., 2014). The regular mode of treatment upto 4-5 years should maximum recovery but not beyond that which enlighted the fact that prolonged medication and gene interactions may re-establishment the concerned gene expression upto certain level but not upto the control level.

Conclusion

From the present study it may conclude that regular treatment mode showed comparatively less damage in the male reproductive function at genetic level in diabetic patients rather than the damage happened under irregular treatment mode. Moreover, this irregular treatment mode is more dangerous to diabetic patients rather than recovery. Therefore this type of study may generate a crucial message to the society regarding the beneficial effect of regular treatment in diabetes.

REFERENCES

Agbaje, I.M., Rogers, D.A., McVicar, C.M., McClure, N., Atkinson, A.B., Mallidis, C., Lewis, S.E. 2007. Insulin dependent diabetes mellitus: implications for male reproductive function. *Hum Reprod*,. 22: 1871-1877.

Aitken, R.J. 1999. The Amoroso Lecture, the human spermatozoon-a cell in crisis?. J *Reprod Fertil*,. 115: 1-2.

Amaral, S., Oliveira, P.J., Ramalho-Santos, J. 2008. Diabetes and the impairment of reproductive function: possible role of mitochondria and reactive oxygen species. *Curr Diab Res*,. 4: 46-54.

Andy, P., Alberti, L.R., Antonio-Barreto-de Melo, M.A., Magalhaes-de-Almida, L. 2009. Relation between diabetes mellitus and male fertility. *Einstein*, 7: 407-410.

Bannister, J.V., Bannister, W.H., Rotilio, G. 1987. Aspects of the structure, function, and applications of superoxide dismutase. *CRC Crit Rev Biochem*., 22: 111-180.

Beers, R.F., Sizer, I.W. 1952. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. *J Biol Chem*,. 195: 133-140.

Bradford, M.M. 1976. A rapid and sensitive for the quantitation of microgram quantitites of protein utilizing the principle of protein-dye binding. *Anal Biochem*,. 72: 248-254.

Ghosh, A., Jana, K., Pakhira, B.P., Ghosh, D. 2016. Antiapoptotic efficacy of seed of Eugenia jambolana on testicular germ cell in experimental diabetic rat: a genomic study. *Andrologia*, 48: 282-292

Goldstein, D.E., Little, R.R., England, J.D., Wiedmeyer, H.M., McKenzie, E.M. 1986. Methods for quantitating glycosylated hemoglobins: high performance liquid chromatography and thiobarbituric acid colorometry: In Clarke WL, Larner, Pohl Sl, editors. Methods in diabetic research. Vol. 2. Clinical methods. New York: John Wiley, pp 475–504.

Klein, R. 1995. Hyperglycemia and microvascular and macrovascular disease in diabetes. *Diab Care*., 18: 258-268.

Kodama, H., Kuribayashi, Y., Gagnon, C. 1996. Effect of sperm lipid peroxidation on fertilization. *J Androl*,. 17: 151–157

Maheshwari, A., Misro, M.M., Aggarwal, A., Sharma, R.K., Nandan, D. 2009. Pathways involved in testicular germ cell apoptosis induced by H2O2 in vitro. *FEBS J*., 276: 870-281.

Mallick, C., Bera, T.K., Ali, K.M., Chatterjee, K., Ghosh, D. 2010. Diabetes-induced testicular disorders vis-à-vis germ cell apoptosis in albino rat: remedial effect of hexane fraction of root of Musa paradisiaca and leaf of Coccinia indica. *J Health Sci*, 56: 641-654.

Marklund, **S.**, Marklund, G. 1974. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *Eur J Biochem*,. 47: 469-474

Moustafa, M.H., Sharma, R.K., Thornton, J., Mascha, E., Abdel-Hafez, M.A., Thomas, A.J., Agarwal, A. 2004. Relationship between ROS production, apoptosis and DNA denaturation in spermatozoa from patients examined for infertility. *Hum Reprod*. 19: 129-138.

O'Neill, J., Czerwiec, A., Agbaje, I., Glenn, J., Stitt, A., McClure, N., Mallidis, C. 2010. Differences in mouse models of diabetes mellitus in studies of male reproduction. *Int J Androl*,. 33: 709-716.

Ray, S., Chatterjee, K., De, D., Ghosh, D. 2014. Bioefficacy of hydromethanolic extract of tuber of Chlorophytum borivilianum (Safed Musli) for the management of male infertility in cyproterone acetate-treated albino rats. *Andrologia*., 46: 659-671.

Ricci, G., Catizone, A., Esposito, R., Pisanti, F.A., Vietri, M.T., Galdieri, M. 2009. Diabetic rat testes: morpholigical and functional alterations. *Andrologia*,. 41: 361-368.

Shrilatha, B. 2007. Early oxidative stress in testis and epididymal sperm in streptozotocin- induce diabetic mice: its progression and genotoxic consequences. *Repord toxicol*., 23: 578-587.

Sokal, R.R., Rohle, F.J. 1997. Introduction to analysis of variance. Biometry. New York: WH Freeman and Company. pp. 179-206

Sun, J.G., Jurisicova, A., Casper, R.F. 1997. Detection of deoxyribonucleic acid in human sperm: correlation with fertilization in vitro. *Biol Reprod*,. 56: 602-607.

Trinder, P. 1969. Determination of blood glucose using an oxidase-peroxidase system with a non-carcinogenic chromogen. *J Clin Pathol*,. 22: 158-161.

Twigg, J., Fulton, N., Gomez, E., Irvine, D., Aitken, R.J. 1998. Analysis of the impact of in- tracellular reactive oxygen species generation on the structural and functional integ-rity of human spermatozoa: lipid peroxidation, DNA fragmentation and effectiveness of antioxidants. *Hum Reprod*,. 13: 1429-1436.

Yan, W., Suominen, J., Samson, M., Jégou, B., Toppari, J. 2000. Involvement of Bcl-2 family proteins in germ cell apoptosis during testicular development in the rat and pro-survival effect of stem cell factor on germ cells in vitro. *Mol and Cell Endocrinol*,. 165: 115-29.

****