

Original Article

Type 2 diabetes mellitus affects male fertility potential

**Amit Kant Singh^{1#}, Shalini Tomar^{2#}, Ajay R. Chaudhari³,
Ramji Singh^{4#} and Narsingh Verma^{5*}**

^{1#} Associate Professor, Department of Physiology,
UP RIMS & R, Saifai, Etawah, India

^{2#} Medical Officer in Obst. and Gynecology,
ESI Hospital, Agra, India

³ Professor Department of Physiology,
MGIMS, Sevagram, Wardha, India

^{4#} Professor Department of Physiology,
AIIMS, Patna, India

⁵ Professor Department of Physiology,
KGMU, Lucknow, India

#Formerly at MGIMS, Sevagram, Wardha, India

Abstract

Objective: Diabetes is a syndrome that affects all the physiological systems of the body, therefore this study was undertaken to compare the seminogram parameters in diabetics and non- diabetics.

Study design: The study was carried out at Male Infertility and Reproductive Physiology unit in the Department of Physiology, MGIMS, Sevagram, Wardha. 25 normozoospermic subjects with type 2 diabetes and 25 normozoospermic non diabetic subjects were recruited in the study. The semen samples were analyzed for sperm concentration, motility and morphology.

Results: In diabetic group the sperm concentration was 24.6 millions/ml with the motility of 52.3% and normal morphology 31.5%, while in non-diabetic group the sperm concentration was 42.7 millions/ml with 63.1% motility and 47.2% normal morphology.

Conclusion: Thus our observations indicate that there is a detrimental effect of type 2 diabetes mellitus on semen parameters.

Introduction

Worldwide, there were approximately 194 million

adults aged 20–79 years with diagnosed diabetes mellitus (DM) in 2003 (with type 2 diabetes accounting for 90–95% of all diagnosed cases), and that number is expected to increase to 333 million over the next 20 years (1).

***Corresponding author :**

Narsingh Verma, Department of Physiology, KGMU, Lucknow, India, Ph. No.: +91-9839064560, E-mail: nsvermakgm@gmail.com
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Oxidative stress, through the production of reactive oxygen species (ROS) has been proposed as the root cause underlying the development of insulin

resistance, B-cell dysfunction, impaired glucose tolerance and type 2 diabetes mellitus (T2DM). It has also been implicated in the progression of long-term diabetes complications, including microvascular and macrovascular dysfunction affecting all the physiological systems of the body (2).

As the oxidative stress is also implicated in male factor infertility, it is hypothesised that type 2 diabetes mellitus has detrimental effect on the semen parameters. Therefore, this study was undertaken to compare the seminogram parameters in type 2 diabetics and non-diabetics.

Materials and Methods

Patient selection

The study was carried out at Male Infertility and Reproductive Physiology unit in the Department of Physiology at MGIMS, Sevagram, Wardha. The study was approved by the institutional ethical committee for conducting research on human beings. 25 subjects with type 2 diabetes and 25 non diabetic subjects showing normozoospermia on the semen analysis (as per WHO protocol) were recruited in the study (3).

Collection of semen sample

Informed written consent was obtained from all the patients involved in this study. The semen samples were collected in a wide mouthed sterile container by masturbation after an abstinence of 3–4 days.

Semen analysis

The semen samples after collection were kept at room temperature. The liquefaction was ascertained at every 5 min till the time of liquefaction. Then the volume was measured and the pH was determined. The relative viscosity was measured as a ratio of the time taken by a known volume of semen to flow through a 20 gauge needle with that of equal volume of distilled water on that day (4).

The semen samples were evaluated by Sperm Quality

Analyzer (SQA-IIB from MES, Israel) for sperm concentration, motility and morphology at room temperature.

Estimation of Malonyl dialdehyde (MDA)

MDA levels were analyzed by Thiobarbituric Acid (TBA) method according to which the semen sample was centrifuged at 3000 rpm for 10 minutes after liquefaction to get the seminal plasma. Then 0.1 mL of seminal plasma was added to 0.9 mL of distilled water in a glass tube, to it 0.5 mL of TBA reagent (0.67 gm of 2 Thiobarbituric acid dissolved in 100 mL of distilled water with 0.5 gm of NaOH and 100 mL of glacial acetic acid) was added and then heated for 1 hour in a boiling water bath. After cooling the tube was centrifuged for 10 minutes at 4000 rpm and the supernatant absorbance was read on a spectrophotometer at 534 nm (5).

Hormone estimation

The plasma concentrations of LH, FSH and testosterone were estimated using the standard radioimmunoassay technique.

Analysis of data

The data in each category were pooled to compute the mean and standard deviation. The statistical differences were evaluated by using one-way ANOVA. A $P < 0.05$ was considered significant.

Results

The mean age of the patients (in years) of type 2 diabetic group was 47.8 ± 3 . and of non diabetic group was 44.3 ± 2.3 .

The pooled data of the groups revealed non significant difference in semen volume, pH, liquefaction time, relative viscosity and plasma concentrations of LH, FSH and testosterone while the sperm concentration, motility, morphology were significantly less in diabetic group as compared to non diabetic group. The MDA level was significantly elevated in diabetic group (Table I).

TABLE I :

<i>Semen parameter</i>	<i>Type 2 diabetics (n=25)</i>	<i>Non diabetics (n=25)</i>
Volume (ml)	2.2±1.1	2.4±0.3
pH	7.8±0.4	7.4±0.7
Liquefaction time (min)	32.7±6.0	30.3±7.4
Relative viscosity to water	7.0±1.9	6.3±1.2
Sperm concentration (million/ml)	24.6±2.1	42.7±4.6*
Motility (%)	52.3±1.3	69.1±3.2*
Morphology (%)	31.5±1.2	47.2±3.7*
MDA (nmol/ml)	2.5±0.6	1.6±0.4*
LH (mIU/ml)Normal range ²⁶ : 1–15	3.9±0.5	4.1±0.3
FSH(mIU/ml)Normal range ²⁶ : 1–15	7.4±1.2	7.1±2.2
Total Testosterone (ng/dl)	346±53.3	362±73.4
Normal range ²⁶ : 300–1100		

Values are Mean±SD.

*P value < 0.05 one way ANOVA.

Discussion

This study was conducted to evaluate the effect of type 2 diabetes mellitus on male fertility potential. The present results reveal that the diabetic subjects have lower semen parameters as compared to non diabetic individuals.

It has been suggested that fluctuating blood glucose concentrations, like those observed during postprandial glycaemic excursions in people with IGT or T2DM, may contribute significantly to oxidative stress perhaps even more so than chronically elevated blood glucose (6). Fluctuating blood glucose level cause the following effects:

1. Decreased nitric oxide availability and generation of ROS. The outcome of this reduction in NO availability is defective endothelial-dependent vasodilatation, leading to microvascular and macrovascular complications (7-11).
2. Increased Markers of Inflammation (12).
3. It has been proposed that T2DM involves a cytokine-mediated acute-phase inflammatory response (12).
4. Elevated blood glucose levels contribute to the glycation of proteins and lipids, resulting in the formation of advanced glycation end products (AGEs) (13).

5. Receptors for AGEs (RAGE) are present in tissues and cell types, including endothelial cells, vascular smooth muscle cells and macrophages (14-16).
6. The binding of AGEs to RAGE leads to the intracellular generation of ROS (17, 18).
7. Lipid peroxidation at cellular level due to increased level of ROS (19).

It is known that oxidative stress affects the testicular function by disruption of germinal cell epithelial division and differentiation.along with the induction of germ cell apoptosis (20, 21).

The mechanisms underlying the apoptosis induction by oxidative stress are not clear. However, they are shown to be due to the involvement of cytokine-induced stresskinase and E-selectin expression in the testicular vascular endothelium (21, 22, 23).

Induction of apoptosis leads to testicular neutrophil recruitment and increases the generation of intra-testicular reactive oxygen species (ROS). ROS in turn, cause peroxidative damage to cell membranes and also activate germ cell apoptosis (810). The rate of phagocytosis by Sertoli cells is also enhanced by increased germ cell apoptosis so as to clear the dying and damaged germ cells (24, 25).

The ROS produces toxic effects at 3 different levels. Firstly ROS activates apoptotic mechanism on gamete cells (21, 22, 23). Secondly suppress the cell division and differentiation directly (20). Thirdly, activates the phagocytic mechanism in Sertoli cells so that damaged and apoptotic cells are phagocytosed (24, 25).

In conclusion as the semen parameters are decreased and MDA level is increased in diabetics as compared to non diabetic group in this study, thus type 2 diabetes mellitus has detrimental effect on male fertility potential. The novelty of this study lies in the fact that it indicates towards a common factor i.e. oxidative stress which is implicated in type 2 diabetes mellitus and also has detrimental effects on semen parameters.

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