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TYPE 2 DIABETES MELLITUS EFFECTS ON SEMEN PARAMETERS AND SEMINAL PLASMA

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ABSTRACT

Background: Diabetes Type 2 is a complex disorder described by an imbalance between insulin resistance and secretion that induce liver glucose output. It has been shown that serum insulin levels are affected by a sperm plasma membrane and acrosome. Therefore, during insulin resistance spermatogenesis changes, diabetic patients detect testicular changes. **Aims**: This research aims to know the effect of diabetic type 2 on some aspects of fertility in men by studying the characteristics of the semen and some biochemical parameters in seminal plasma. **Methods:** This study was achieved at the Center for Endocrinology and Diabetes Specialists in Maysan province from February to November 2018 and included 45 men (30 diabetic and 15 healthy in the control group) aged 30 to 59 years. The patients were divided according to age into two groups, the first (30-39) and second (40-59) years, also divided by the duration of diabetes into two groups, the first (1-5) and second (6-10) year. **Results:** The pH of semen in the second age group (30-49 years) group and first duration (1-5 years) group were significantly decreased ($P < 0.05$) in diabetes compared with the control group. Volume and viscosity did not have significant differences in patients compared to the control following the age and duration of diabetes. Liquefaction only in the first age (30-39 years) group significantly increased ($P<0.05$) compared to the control. The concentration of sperm, progressive motility, non-progressive and normal morphology decreased ($P < 0.05$) significantly. While the sluggish, dead, and abnormal morphology significantly increased ($P < 0.05$) in all diabetes groups compared with the control. The fructose and alkaline phosphatase values in the seminal plasma were not differing significantly in patients compared with the control. Zinc and glutathione values decreased significantly ($P < 0.05$) compared with control in each age and duration of diabetes. **Discussion:** Insulin stimulates the Ledying cell function, defect insulin effect on spermatogenesis. Impaired sperm motility in a patient with D.M. might be attributed to many reasons, such as increased ROS level, altered mitochondria DNA, and decreased epididymal products. **Conclusion:** our measurement indicates that there is an effect of type 2 diabetes mellitus on semen parameters and seminal plasma biochemical parameters.

Keywords: *Type 2 Diabetes, Semen, Parameters*.

1. INTRODUCTION

Diabetes mellitus (D.M.) is a main affront to universal public health, and it comprises various etiology of diseases characterized by high blood glucose (Dragana *et al.,2015*). Diabetes Type I is caused by autoimmune devastation of pancreatic β cells, which produce insulin, and insulinogenic with resultant high blood glucose. It is diagnosed during childhood and requires exogenous insulin to exist (Zipitis andAkobeng,2008**).** Diabetes Type II is a complex disorder described by an imbalance between insulin resistance and insulin secretion that induce liver glucose output by preventing glycogen formation and stimulating glycogenolysis and gluconeogenesis (H.M., K., 2015).

It has been shown that serum insulin levels are affected by the sperm plasma membrane and acrosome (Silvestroni *et al*., 1992). Therefore, during insulin resistance or insulin deficiency, spermatogenesis changes and biopsies of diabetic patients detect testicular changes (Cameron *et al*.,1985). D.M. may affect male reproductive function at numerous levels due to its effects on the endocrine control of spermatogenesis or by weakening penile erection and ejaculation (Bener *et al.,*2009). Diabetes is associated with reproductive retrogradation in

both men and women. About 90% of diabetic persons suffer from a decline in sexual function, such as decreased libido, fertility, and impotence (Omolaoye and Du Plessis, 2018). D.M. causes an effect on the epididymis, with a negative effect on spermatozoon crossing. A varied mechanism is made as the case for the spermatozoon damage discovered in patients with diabetes, and these comprise endocrine disorders, neuropathy, and increased oxidative stress. Also, type II DM connected with hypothalamic-pituitary-gonadal (HPG) axis inhibition was a decrease in testosterone and another sex hormone (Al-Aaraji, 2016). So, the research aims to know the effect of diabetes mellitus type 2 on some aspects of fertility in men by studying the macroscopic and microscopic characteristics of the semen.

2. MATERIALS AND METHODS

2.1. Materials

The materials used in this study were listed as follows:

1- Alcohol methyl
2- Centrifuge
3- Container cup
4- EILSA
5- Glass slide and cover slips
6- Hemocytometer
7- Plain tube(10 ml)
8- Refrigerator
9- Spectrophotometers
10- ALP kit
11- Fructose kit
12- GSH kit
13- Zinc kit

2.2. Methods

2.2.1. The subjects

This study was achieved at the Center for Endocrinology and Diabetes specialist in Maysan province, including 60 men (45 diabetic and 15 healthy as the control group) aged 30 to 49 years. The number was divided by age into two groups, the first (30-39) years and second (40-49) years, also divided by the duration of diabetes into two groups, the first (1-5) years and second (6-10) years.

2.2.2 Semen collection

The ejaculates were collected after an abstinence period of (3-5days). In a sterile, no toxic, disposable container and transported to the laboratory within 30 minutes after ejaculation.

2.2.3 Semen examination

The macroscopic examination of semen included liquefaction, appearance, volume, viscosity, and pH measurement, according to WHO 2010 manual (WHO, 2010).In addition, the Microscopic examination of semen included concentration, motility [Progressive motility(A), Non-progressive motility (B), Sluggish (C), and Immotility (D)] and Sperm morphology (%) estimation according to (Tocci, and Lucchini, 2010).

2.2.4 Seminal plasma analysis

Seminal plasma preparation and Storage by Centrifugation of the semen samples for 15 minutes at 2600 rpm and rapidly recovering of the seminal supernatant plasma and placed to freeze at -20 °C to be measured biochemical parameters in seminal plasma (Vasan, 2011). The Biochemical parameters in Seminal plasma, including:

2.2.4.1 Fructose

In the presence of ATP, D- fructose transforms from Esochinase (E.K.) in fructose -6 phosphate. The fructose -6 phosphate is transformed from phosphor-Gluco-isomerase (PGI) in glucose -6 phosphate, which is transformed in 6-phosphgluconate from G6P-DH with the formation of NADPH. NADPH formed in this reaction causes an increase in absorbance at 340 nm. The principle of the kit is according to Nagasaka *et al.* (1988).

2.2.4.2 Zinc measurement

At room temperature, the zinc reacts with the chromogen present in the reagent, giving a colored complex measured by spectrophotometers with a strength proportional to the zinc concentration in the sample. The principle of the kit is according to Makino(1991).

2.2.4.3 Alkaline phosphatase (ALP) and Glutathione (GSH)

This kit uses enzyme-linked immune sorbent assay (ELISA) based on biotin double antibody sandwich technology to assay human alkaline phosphatase(ALP) and human glutathione (GSH). Add alkaline phosphatase(ALP) and glutathione (GSH) to wells that are pre-coated with glutathione (GSH) monoclonal antibody and then incubate. To wells pre-coated with alkaline phosphatase(ALP) monoclonal antibody and then incubated. After incubation, add anti-ALP antibodies labeled with biotin to unite with streptavidin-HRP, which forms the immune complex. Remove unbound enzymes after incubation and washing, then add substrates A and B. The solution will turn blue and change to yellow with the effect of acid. The shades of the solution, human alkaline phosphatase (ALP) concentration, and Human glutathione (GSH) are positively correlated.

2.3 Statistical analysis

The data obtained during this study were analyzed by one-way ANOVA using a statistical package for social science (SPSS) (Bryman and Cramer,2004). The data are presented as mean ± S.E. The difference was considered to be significant at P<0.05.

3. RESULTS AND DISCUSSION

3.1. Results

3.1.1 Semen parameters (According to age)

The pH in the second group decreased significantly (P <0.05) compared to the control group, and the control group did not differ significantly from the first group. In contrast, the first group did not differ significantly (P<0.05) compared to the second group. No significant differences (P<0.05) in the semen volume in all groups. The liquefaction in the first group was significantly increased(P <0.05) compared to the control group. It did not differ significantly from the second group, while the second group did not differ significantly (P<0.05) compared to the control group. There are no significant differences (P<0.05) in the viscosity of all groups (Table 1).

The concentration of sperm in the first and second groups decreased significantly (P <0.05) compared to the control group. However, the first and second groups did not differ significantly (P<0.05) between them. The progressive motility (A) of sperm in the first and second groups decreased significantly (P <0.05) in comparison with the control group. However, the first and second groups did not differ significantly (P<0.05) between them (Table 1). The Non-progressive motility (B) of sperm in the first and second groups decreased significantly (P <0.05) in comparison with the control group. However, the first and second groups did not differ significantly (P<0.05) between them. The Sluggish motility (C) of sperm in the second group was increased significantly (P-<0.05) in comparison with the control but did not differ significantly from the first group. At the same time, all groups increased significantly (P<0.05) compared to the control group. The Dead motility (D) of sperm in the first and second groups was increased significantly (P<0.05) in comparison with the control group. However, the first group did not differ significantly (P<0.05) in comparison with the second group (Table 1). Normal morphology of sperm in the first and second groups decreased significantly (P <0.05) compared with the control group. However, the second group did not differ significantly (P<0.05) compared to the first group. Abnormal morphology of sperm in the first and second groups was increased significantly (P <0.05) compared to the control group. Also, the first group differs significantly (P<0.05) in comparison with the second group (Table 1).

No significant variation was recorded in the value of fructose in the first and second groups compared to the control. The zinc values in the first and second groups decreased significantly (P<0.05) in comparison with the control group, while the first and second groups did not differ significantly (P<0.05) between them. No significant variation was recorded in the value of ALP in the first and second groups compared to the control. The GSH values in the first and second groups decreased significantly (P<0.05) compared to the control group. However, the first and the second group did not differ significantly (P<0.05) between them (Table 2).

3.1.2 Semen parameters (According to the duration of T2DM)

The pH in the first and second groups decreased significantly (P<0.05) compared to the control group. In contrast, the first and second groups did not differ significantly (P<0.05). The

volume no significant differences (P<0.05) in the semen volume in all groups. The liquefaction and viscosity in the first, second, and control groups did not show significant differences(P<0.05) (Table 3).

The concentration of sperm in the first and second groups decreased significantly (P<0.05) compared to the control group. However, the first and second groups did not differ significantly (P<0.05) between them. The motility (A) of sperm in the first and second groups decreased significantly(P <0.05) in comparison with the control group. However, the first and second groups did not differ significantly (P<0.05) between them. The motility (B) of sperm in the first and second groups decreased significantly (P <0.05) in comparison with the control group, but the first and second groups did not differ significantly (P<0.05) between them. The motility(C) in the second group was increased significantly (P <0.05) in comparison with the first and the control groups.

Also, the first and second groups differed significantly (P<0.05). The motility (D) of sperm in the first and second groups was increased significantly (P <0.05) in comparison with the control group. Also, the first and second groups differed significantly (P<0.05). Normal morphology of sperm in the first and second groups decreased significantly (P <0.05) compared to the control group. However, the first group did not differ significantly (P<0.05) compared to the control group. Abnormal morphology of sperm in the first and second groups was increased significantly (P <0.05 in comparison with the control group, but the first group did not differ significantly(P<0.05) compared to the second group (Table 3).

No significant variation was recorded in the value of fructose in the first and second groups, respectively, compared with the control. The zinc values in the first and second groups decreased significantly (P<0.05) compared to the control group. However, the first and second groups did not differ significantly (P<0.05) between them. No significant variation was recorded in the value of ALP in the first, second, and control groups. The GSH values in the first and second groups decreased significantly (P<0.05) compared to the control group. Also, the first and second groups did not differ significantly (P<0.05) between them (Table 4).

3.2. Discussions

3.2.1 Semen parameters (According to age)

The study recorded that pH and liquefaction in the semen of diabetes patients decreased and increased significantly. Mohammad and Ameen (2021) found no significant variation in the pH of semen values between diabetic(7.72 ± 0.21) and control(7.64 ± 0.13). The epididymis contains acetic acid, which spreads to the sperm to reduce the degree of pH (Sircar, 2008). And increased significantly in liquefaction in patients compared with control may be due to the absence of activity of fibrinolytic enzymes found in seminal fluid(Pilch and Mann,2006). The result recorded that the concentration, progressive, no progressive motility, and normal morphology are significantly decreased, while the increase in sluggish motility, dead and abnormal morphology. This result agrees with (Garcia-diez *et al.*,1991; Mohammad and Ameen,,2021), who found a decrease significantly in sperm concentration, sperm motility, and sperm morphology. Also, Bhattacharya *et al*. (2014) Found a decrease significant in percentage motility and normal morphology compared with the control. On the other hand (Ali *et al.*,1993) reported increased sperm concentration in men with insulin-dependent diabetes mellitus (IDDM) and non-insulin-dependent mellitus (NIDDM) with neuropathy, compared with control. Also, Mohammad and Ameen (2021) observed sperm concentration in males with diabetes mellitus and obesity significantly different from the control.

The germ cell apoptosis and spermatogenesis disorders in D. M. are related to local autoimmune damage. Insulin stimulates the Ledying cell function, whereas defective insulin causes defective spermatogenesis (Perrard-Sapori *et al*., 1987). Sperm cells are susceptible to oxidative stress, and sperm cell contain high concentrations of polyunsaturated fatty acid in the membrane(John Aitken *et al*., 1989). D. M. also provokes injurious blood testes barrier alteration, possibly responsible for spermatogenesis disruption (Alves *et al*., 2013). The study shows that motility was significantly lower in inpatient D. M. than in control. Impaired sperm motility in patients with D. M. might be attributed to many reasons, such as increased ROS level, altered mitochondria DNA, And decreased epididymal products (Singh *et al*., 2009; Niwas Jangir and Chand Jain,2014). D. M. leads to a marked reduction in fecundity by altering the normal

morphology of sperm cells and sperm parameters are affected in cases of D. M. Also, increased oxidative stress was hurtful to sperm morphology and is considered a major factor of low normal sperm morphology in D. M. Also increased lipid peroxidation in patients with diabetes is associated with low normal sperm morphology and increased abnormal morphology (LaVignera *et al*., 2012). Abnormal sperm would be a source of superoxide anions that bind with zinc in seminal plasma and thus decrease the zinc levels (Colagar *et al*., 2009).

The study showed a significant reduction in Zinc and GSH in diabetes compared with control, while the fructose and ALP did not reach a significant level. Our result agrees with many studies that reported the level of zinc in seminal plasma decreased significantly in patients of men diabetic in compared with control (Praveeena *et al.*, 2013; Ozturk *et al*., 2013; Ghasemi *et al*., 2016). Zn microelement is essential for male fertility, and their deficiency impedes spermatogenesis and is a reason for sperm abnormalities, and has a negative effect on serum testosterone concentration (Allouche-Fitoussi, and Breitbart, 2020). Low zinc in men may be caused prostate cancer and infertility and is linked to low libido (Mohamed, 2010). The study agrees with de Oliveira *et al*. (2016), who noted a significant reduction in GSH in diabetic rats compared to the control.

Higher levels of GSH in seminal plasma may protect against oxidative damage and progress sperm characteristics (motility and morphology) (Atig *et al*., 2012). Hyperglycemia raises O.S. by increasing ROS formation and altering the normal oxidation–reduction state. Several mechanisms contribute, such as an increased polyol pathway flux, Increased intracellular formation of advanced glycation end (AGEs) products, activation of protein kinase C, and overproduction of superoxide in the mitochondria (Brownlee, 2001). Diabetes induces alteration in the activity of the enzyme glutathione peroxidase and reductase; these enzyme found in all cell that metabolizes peroxide to water and converts glutathione disulfide back into glutathione, so alteration in their levels make cell lie down to oxidative stress and cause cell injury (Asmat *et al*., 2016).

3.2.2 Semen parameters (According to the duration of T2DM)

A study by Padrón *et al*. (1984) and Condorelli *et al*. (2018) noted a decrease significantly in sperm concentration, sperm motility, and sperm morphology compared to control with time-progressive duration diseases. Decreases in sperm concentration in diabetes mellitus compared to control may be due to low FSH and L.H., which are responsible for spermatogenesis (Maneesh *et al*., 2006). Sperm cells are susceptible to oxidative stress, and sperm cells contain high concentrations of polyunsaturated fatty acid in the membrane, increasing with progressive time duration (John Aitken *et al*., 1989).

The decreased motility observed in D. M. patients might be attributed to increased ROS levels and altered mitochondrial DNA (Niwas Jangir and Chand Jain, 2014). The author (Sikka, 1996) found a high level of reactive oxygen species in abnormal sperm compared to normal sperm. The increase in abnormal sperm may be due to increased oxidative stress and an increase in reactive oxygen species (ROS). On the other hand, decreased motility may be due to increased oxidative stress, which is accompanied by an increase in ROS, which leads to decreased mitochondrial membrane potential (MMP) that is associated with decreased motility of sperm (Condorelli *et al*., 2018; Irigoyen *et al.,* 2022). This study shows significant differences in Zinc and GSH between patients with diabetes and control and did not differ significantly in fructose, and ALP values (Adewole *et al.,* 2007) mentioned that Zinc and GSH decreased significantly in diabetes induce rats along with time-progressive diabetes. Diabetes induces a change in antioxidant agents, which play an important role in removing reactive oxygen species (ROS), and a decrease of antioxidants, exposing the cell to defects (Asmat *et al*., 2016; Amaral *et al.,*2006).

Insufficient antioxidant protection renders spermatozoa vulnerable to oxidative damage(Eskiocak *et al.,*2005). Antioxidants improve insulin sensitivity by reducing oxidative stress and insulin resistance (Udupa *et al*.,2012). Low zinc in men may be caused prostate cancer and infertility and is linked to low libido (Mohamed,2010). Zinc improves sperm quality as a membrane stabilizer and, as a component of superoxide dismutase, prevents sperm apoptosis and sperm DNA fragmentation (Omu *et al*., 2014).

4. CONCLUSIONS

It was concluded that there is an effect of type 2 diabetes mellitus on semen parameters and seminal plasma biochemical parameters.

Especially on the parameters of semen (decreased pH, concentration of sperm, mortality A and B, normal morphology) and low values of the zinc and GSH in seminal plasma.

5. DECLARATIONS

5.1. Study Limitations

The study is limited to the sample size and the analysis conducted.

5.2. Acknowledgements

We would thank Dr. Assad Yahia for assisting in the statistical analysis. Great thanks to the patients and control individuals for participation and facilitating in the completion of this study.

5.3. Funding source

The authors funded this research.

5.4. Competing Interests

There are no conflicts of interest.

6. HUMAN AND ANIMAL-RELATED STUDIES

6.1. Ethical Approval

Permission to conduct this study was issued by the health institutional Center for Endocrinology and Diabetes specialist in Maysan Province in order to facilitate mission number 332 on 27.11.2017, and a public health technician carried out the semen sampling from patients and control.

6.2. Informed Consent

A public health technician References carried out the semen sampling from patients, and control and verbal consent were taken from the participants

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Table 1: parameter of semen in diabetic mellitus type 2 and control (According to age) (mean ± S.E.)

Macroscopic examination of semen					
Parameters		**Control (30-59)year**	**First group (30-39)year**	**Second group (40-49)year**	LSD
P.H.		a 8.00±0.00	a b 7.84±0.08	b 7.80±0.60	0.17
Volume(ml)		3.53±0.21	3.13±0.37	3.20±0.38	N.S.
Liquefaction (min)		b 29.33±0.66	a 34.00±2.72	a b 30.00±0.66	4.62
Viscosity		1.00±0.03	1.05±0.05	1.08±0.09	N.S.
Microscopic examination of semen					
Concentration (×10^6/ml)		a 62.46±4.20	b 27.66±5.72	b 37.66±3.41	12.96
Motility %	Progressive(A)	a 31.33±2.46	b 5.33±1.72	b 8.00±1.52	5.54
	Non-progressive(B)	a 34.66±1.24	b 14.33±2.42	b 16.33±1.96	5.53
	Sluggish (C)	b 15.00±1.54	a 30.33±4.21	a 30.66±2.84	8.73
	Dead (D)	b 19.00±1.90	a 50.00±8.35	a 45.00±4.90	16.24
Morphology %	Normal	a 82.00±1.52	b 46.00±7.71	b 59.33±3.34	14.06
	Abnormal	b 18.00±1.52	a 54.00±6.51	c 40.66±3.34	12.30

Table 2: Biochemical parameters of semen plasma in diabetic mellitus type 2 and control (According to age) (mean ± S.E.)

Parameters	Control (30-59) year	First group (30-39) year	Second group (40-49) year	LSD
Fructose(mg/ml)	5.51±0.94	8.11±1.86	9.34±1.60	N.S.
Zinc(µg/ml)	a 162.93±9.18	b 141.86±5.38	b 132.00±3.47	18.42
ALP(IU/L)	229.70±24.82	188.48±17.77	176.91±25.42	N.S.
GSH(ng/L)	a 17.64±0.82	b 13.88±0.92	b 13.87±0.64	2.29

Table 3: Parameters of semen in diabetic type 2 and control (According to duration)

Macroscopic examination of semen					
Parameters		**Control**	**First group (1-5) year**	**Second group (6-10)year**	**LSD**
P.H.		a 8.00±0.00	b 7.76±0.07	ab 7.90±0.60	0.16
Volume (ml)		3.53±0.21	3.15±0.32	3.20±0.38	N.S.
Liquefaction (min)		29.33±0.66	34.00±2.72	30.00±0.66	N.S.
Viscosity		1.00±0.03	1.05±0.05	1.09±0.09	N.S.
Microscopic examination of semen					
Concentration (×10^6/ml)		a 62.46±4.20	b 35.05±4.53	b 28.54±4.98	13.18
Motility %	**Progressive(A)**	a 31.33±2.46	b 6.97±1.72	b 7.72±1.23	5.26
	Non-progressive (B)	a 34.66±1.24	b 15.39±1.81	b 17.74±1.23	4.56
	Sluggish (C)	c 15.00±1.54	b 22.63±2.39	a 35.90±2.76	6.53
	Dead (D)	c 19.00±2.90	a 55.00±4.35	b 38.63±2.86	10.26
Morphology %	**Normal**	a 82.00±1.52	b 60.26±3.94	b 51.81±3.83	9.78
	Abnormal	b 18.00±1.52	a 39.73±3.94	a 48.18±3.83	9.78

Table 4: Biochemical parameters of semen plasma in diabetic mellitus type 2 and control (According to duration) (mean ± S.E.)

Parameters	Control	First group (1-5)year	Second group (6-10)year	LSD
Fructose(mg/ml)	5.51±0.94	8.16±1.63	9.56±1.81	N.S.
Zinc(µg/ml)	a 162.93±9.18	b 142.52±5.38	b 140.09±5.49	19.97
ALP(IU/L)	229.70±24.82	186.75±18.08	226.60±25.90	N.S.
GSH(ng/L)	a 17.64±0.82	b 14.47±0.72	b 15.81±0.55	2.11