To study the impact of diabetes on male fertility of Bhagalpur district of Bihar.

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Abstract: The goal of this study was to see how diabetes affected male fertility in the Bihar district of Bhagalpur. This cross-sectional study included 40 men from infertile couples, and their sperm samples were collected along with their general information. Sperm concentration, count, motility, and morphology were observed as general characteristics. The sperm DNA fragmentation index was calculated using the sperm chromatin dispersion (SCD) assay (DFI). To classify groups as normal (DFI 30%) or abnormal (DFI > 30%), a DFI 30% threshold was used. Diabetes was found to be significantly associated with sperm motility, morphology, and DFI. There was, however, no correlation with sperm count. In this study, 2 sperm samples out of 40 had abnormal motility (32% progressive motility) and 7 sperm samples out of 40 had abnormal morphology (Teratozoospermia). We found no link between DFI and motility or morphology. The sperm DNA fragmentation index did not correlate well with other sperm parameters. As a result, a sperm DNA fragmentation assay should be performed as an additional step in determining sperm fertility.

Keywords: Sperm, DNA fragmentation, diabetes, motility, morphology, DFI, SCD, Male fertility and semen parameters.

Introduction:

Following the consumption of food, the pancreas releases insulin to control blood sugar levels. When the body is unable to take glucose into its cells and use it for energy, excess sugar accumulates in the bloodstream, resulting in diabetes. Type 1 diabetes is an autoimmune disorder in which the insulin-producing cells are destroyed. Pre-diabetes occurs when the body is unable to properly process sugars, resulting in sugar levels that are normally high but not high enough to be classified as type 2 diabetes. When blood sugar levels are not properly controlled, they rise and lead to type 2 diabetes. This occurs when the pancreas does not produce enough insulin to control blood sugar levels, or when the body becomes immune to insulin and it no longer works.

Men with pre-diabetes (blood sugar levels higher than normal) have lower testosterone levels, more damage to sperm DNA, and sometimes azoospermia (absence of sperm). Low sperm count, erectile dysfunction, and libido can all be symptoms of hypogonadism (low testosterone levels) (decreased sex drive). Diabetes has also been linked to problems with ejaculation and balanitis (inflammation of the tip of the penis). Diabetes Mellitus (DM) is a group of diseases characterized by an excess of sugar in the body. This causes long-term organ damage, resulting in dysfunction and failure. The process by which the food we eat is converted into glucose for transport through the blood cells is known as glucose metabolism. When enough glucose accumulates around the cells, the pancreas produces insulin. The metabolism of glucose is required for the production and development of mature spermatozoa. It is also necessary for the survival of basic cells and aids in the motility and hyper activation of human sperm. Diabetes can have a negative impact on sperm quality, DNA integrity, motility, and seminal plasma components (the fluid from secretions of organs or tubules in the seminal tract). To ensure energy supplies, spermatozoa metabolize a variety of substrates or pathways. Any vulnerabilities in these testicular metabolic pathways result in altered sperm metabolism, lowering sperm quality.

According to M. I. Agbaje and his colleagues in the year 2007 suggested that diabetes is associated with increased sperm nuclear and mitochondrial DNA damage that may impair the reproductive capability of these men. In the year 2017, Jing-Zhen Zhu and his co-researchers put forward that, in patients with diabetes, the prevalence of primary and secondary infertility was significantly higher than in patients without diabetes. One of the major factors contributing to male DM individuals' subfertility and/or infertility is poor sperm quality due to abnormal sperm parameters such as concentration, motility, morphology, and DNA fragmentation *(Zhu. Zhen-Jing et. al, 2017).* They also claimed that DM patients had lower sperm volume, sperm concentration, total sperm motility, progressive sperm motility, and normal sperm morphology than non-diabetic controls. Furthermore, DM patients had significantly higher sperm DNA fragmentation than non-diabetic controls.

Guo-lian Ding and colleagues proposed in 2015 that either type 1 or type 2 diabetes could have a negative impact on male fertility, specifically sperm quality, such as sperm motility, sperm DNA integrity, and seminal plasma ingredients. Diabetes may affect epigenetic modification during sperm spermatogenesis, and these epigenetic dysregulations may be inherited through the male germ line and passed on to more than one generation, increasing the risk of diabetes in offspring *(Ding Guo-Lian et.al, 2015).* In their findings, Rosita A. Condorelli and fellow researchers found that Diabetic patients were more likely to become infertile, and the pathophysiological mechanisms of damage differed between DM1 and DM2. Diabetic patients' conventional sperm parameters are worse than controls. The DM2 induced an inflammatory state with increased oxidative stress, which resulted in decreased sperm vitality and increased sperm DNA fragmentation. DM1 altered epididymal voiding, resulting in low ejaculate volume and mitochondrial damage, which reduced sperm motility. These findings and evidences support the contention that DM could be regarded as a cause of male infertility, implying that prevention of diabetic disease in DM2 and seminal parameter monitoring in DM1 could prevent fertility decline in these patient groups *(Condorelli A. Rosita et. al, 2018).*

The lower reference limits for semen analyses have been changed by the WHO. The acceptable 5th percentile is represented by the parameters listed below (TG Cooper, et. al. 2010):

- Volume: 1.5 millilitres (95 percent CI: 1.4-1.7)
- Sperm concentration: 15 million spermatozoa per millilitre (95 percent confidence interval: 12-16).
- Total number of spermatozoa per ejaculate: 39 million (95 percent CI: 33-46)
- Morphology: 4% normal forms (95 percent confidence interval: 3-4), adopting the "strict" Tygerberg technique.
- Vitality: 58 percent of the population is alive (95 percent CI: 55-63)
- Progression of motility: 32% (95 percent CI: 31-34)
- Total motility (progressive + non-progressive): 40% (95 percent CI: 38-42)

Bhagalpur is a city in the Indian state of Bihar, located on the southern bank of the Ganges. It is the third-largest city in the state and the administrative center for the Bhagalpur district and division. Silk City is a significant educational, commercial, and political hub that has been identified for development through the Smart City programme, which is government-industry collaboration. The main crops grown on the Gangetic plains that surround the city are rice, wheat, maize, barley, and oilseeds. It is located on the Ganga basin plains at a height of 141 feet above sea level. It covers an area of 2569.50 square kilometers. It is situated between latitudes 25o 07' and 25o 30' north and longitudes 86o 37' and 87o 30' east.

Methodology-

Data collection- A cross-sectional study was conducted on 20 women from infertile marriages with diabetic husbands. Data was retrieved from patients through personal interviews and counselling after records were checked for studies that were eligible. The study included men from infertile couples diagnosed with infertility according to WHO guidelines, with semen analysis and halosperm test results. All of the patients volunteered to take part in the study**.**

Clinical approaches- As general information, age, occupation, geography, duration of infertility, history of any internal ailments, food habits, lifestyle, diabetic or non-diabetic, address, and contact number were all recorded.

Laboratory procedure-

- **Semen analysis-** The sperm samples of the concerned and counselled patients were obtained and examined. Under the microscope, sperm motility, vitality, sperm concentration, sperm count, sperm morphology, head defect, midpiece defect, tail defect, pus cell, and agglutination were all analyzed.

  Sperm concentration- A Makler chamber was used to test sperm concentration and motility. When the cover slip is placed on the quartz pins in the Makler chamber, the volume enclosed between the two layers is exactly 1 million parts of ml *(W Maya-Cardona et. al, 2008).*

  Sperm count- The number of spermatozoa counted in each of the grid's ten square strips corresponds to their concentration in millions per millilitre. The sperm in each of the three alternate columns was counted, and the average value was determined *(E Zuvela et. al, 2020).*

  Motility- The sperm motility parameter was determined by manual counting under a compound microscope at 100x total magnification. Using the formula, the percentages of progressive (PR), non-progressive (NPR), and non-motile were calculated *(Nguyen, T et. al. 2019).*

$$\frac{PR + NPR}{\text{Total count}} * 100$$

  Morphology- Morphology was calculated using Eosin-nigrosin stained smears, and 200-300 sperm were counted in different focus at 100X. Staining a seminal smear and examining it under a 100x magnification allows for the quantitative examination of normal and aberrant sperm morphological forms in an ejaculate. A nigrosin-eosin stain is commonly used because it is effective, simple, and a "live-dead" stain that allows for the evaluation of membrane integrity as well as morphology. The nigrosin stain creates a black background against which the sperm appear as light-colored objects. Normal live sperm do not take up the eosin stain and appear white due to the intact plasma membrane, whereas dead sperm appear pinkish due to the lack of membrane integrity *(Gacem S. et. al. 2021).*

DNA fragmentation test- Using a cryolabs sperm chroma kit, all sperm was tested for fragmented DNA (SAR healthline). This halosperm test is based on the sperm chromatin dispersion (SCD) *(Fernández et al. 2005)* approach, which includes a controlled DNA denaturation process to facilitate the subsequent removal of the protein contained in each spermatozoan. Normal spermatozoa produce halos in this way, which are produced by DNA loops at the sperm's head and are absent in those with damaged DNA.

In culture medium, the sperm was diluted until it reached a maximum concentration of 20 million spermatozoa per militre. Aliquots of 0.2ml of fresh sample semen were diluted in medium to obtain sperm concentrations of 5-10 million per militre. For 5 minutes at 90 degrees, the agarose gel was melted in the sperm chroma warmer 1. The agarose was transferred to sperm chroma warmer 2 and kept warm at 37 degrees for 5 minutes. A 25 microlitre amount of sperm was mixed thoroughly with agarose. The sperm cell suspension was immediately deposited onto the pre-heated slides and covered with a cover slip to avoid the formation of air bubbles. The slides were kept at 4 degrees for 5 minutes. The slides were then carefully removed. The slides were then incubated horizontally in solution A for 7 minutes (denaturation solution). The slides were then horizontally incubated in lysis solution for 25 minutes. The slides were immersed in distilled water for 5 minutes. Following that, the slides were immersed in 70% ethanol for 2 minutes, followed by 90% ethanol for 2 minutes, and finally 100% ethanol for 2 minutes. After that, a horizontal layer of stain was applied and left for another 15-20 minutes after mixing solution C and solution D. (1:1). The stain was decanted and rinsed gently with distilled water before drying at room temperature. The slides were examined using a bright field microscope with a 20X or 40X objective. The resulting halos images were highly contrasted and can be thoroughly examined using standard methods. There are five SCD patterns that can be used, as shown below-

• The massively haloed sperm cell.
• The medium haloed sperm cell.
• The small halo that encircled the sperm cell.

According to the manufacturer's instructions, 300-500 spermatozoa were counted, and those with DNA fragmentation were identified using-

$$\text{DFI (\%)} = 100 \times \frac{\text{No. of spermatozoa with fragmented DNA}}{\text{No. of spermatozoa counted}}$$

Result-

Patients	**Age**	**Semen profile**			
		Sperm count (million/ml)	**Motility rate (%)**	**Morphology (%)**	**DFI report**
1	26 years	18	78	5	28%
2	26 years	38	54	9	26%
3	28 years	9	11	1	60%
4	29 years	4	50	3	36%
5	29 years	36	72	5	29%
6	29 years	40	83	14	19%
7	30 years	28	75	6	36%
8	30 years	31	71	12	21%
9	30 years	43	63	4	24%
10	30 years	26	81	5	28%
11	31 years	30	70	11	22%
12	31 years	26	81	8	24%
13	32 years	43	84	7	35%
14	32 years	6	50	2	22%
15	33 years	38	65	6	21%
16	33 Years	31	71	7	42%
17	34 years	36	81	11	34%
18	34 years	36	86	7	25%
19	34 years	30	87	4	26%
20	35 years	43	72	5	15%
21	35 years	28	40	12	31%
22	36 years	41	78	10	69%
23	37 Years	28	82	7	35%
24	37 Years	26	60	8	24%
25	37 Years	36	61	5	28%
26	37 Years	34	59	9	27%
27	37 years	10	30	4	76%
28	37 years	40	60	4	18%
29	38 years	19	79	5	14%
30	39 years	34	44	4	24%
31	39 Years	9	56	2	29%
32	40 Years	15	40	3	29%
33	40 years	35	89	6	6%
34	41 years	13	62	4	19%
35	42 Years	21	40	9	25%
36	42 years	23	65	3	38%
37	43 Years	36	78	4	39%
38	44 Years	35	83	6	37%

39	45 years	24	35	7	19%
40	49 Years	28	36	3	33%

Discussion-

The study group included a total of 40 infertile couples. The general characteristics and DFI results are shown in Table 1. There are 14 couples with DFI greater than 30% and 26 couples with DFI less than 30%, with a maximum DFI of 6% and a minimum DFI of 76%. However, the diabetes or sugar level of the respective patients had no effect on sperm count. Only four men were found to have sperm counts lower than 15 million/ml (as per WHO lower reference limits for sperm analysis).

Diabetes is strongly linked to motility, morphology, and DFI. Many previous year research papers also suggested that diabetes has a greater impact on sperm quality rather than sperm count. Only 2 out of 40 sperm samples showed abnormal motility (32% progressive motility) rate (Asthenozoospermia) in this study, while the remaining 38 sperm samples showed normal sperm motility. 7 out of 40 semen samples also showed abnormal sperm morphology (Teratozoospermia) as per lower reference limit for semen analysis provided by WHO, 2010. However, in the year 2018, Rosita A. Condrorelli, et al showed in their data that there was a decrease in sperm progressive motility in patients with diabetes (*Condrorelli. A Rosita, et al, 2018)*. In addition, in 2017, Zhu Zhen-Jing and his colleagues proposed that diabetes Mellitus had a negative effect on sperm morphology *(Jing Zhen- Zhu, et al, 2017).* Increased ROS levels, altered mitochondrial DNA, or abnormal glucose metabolism could all explain the decreased motility *(GL Ding, et al, 2015)*. Specific conditions, such as workplace-related exertion and hypertension, impair sperm morphology *(Eisenberg, M. L, et al, 2015)*. Diabetes-related oxidative stress may be to blame for the high DNA fragmentation. Several studies have also found that oxidative damage to sperm DNA is linked to higher levels of reactive oxygen species (ROS) in diabetics *(Mazzilli. F, et al, 1994).*

Beyond concerns about impotence, the effects of diabetes on human male reproductive function have been largely ignored. This is significant from a clinical standpoint, especially given the overwhelming evidence that sperm DNA damage impairs male fertility and reproductive health. More research is needed to determine the precise nature of this damage, the underlying aetiological mechanisms, and its clinical significance.

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