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Research Article

Male Infertility in Meghalaya

Prevalence of Male Infertility in Meghalaya, India. A Cross-Sectional Study

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Abstract: Infertility is a condition of the reproductive system that can affect either the male or the female and is characterized by a failure to achieve pregnancy after 12 months or more of consistent sexual interaction that is not protected. Within the scope of the present study, we aim to look into the prevalence of male infertility with an emphasis on Sperm DNA fragmentation over its importance in conjunction with a semen analysis. Our objective is to confirm the diagnosis of male infertility not be solely dependent on semen analysis alone; however, confirmatory diagnostic test such as Sperm DNA fragmentation needs to be incorporated into the routine investigation. This study marks the first documented report on male infertility in Meghalaya. Using the Makler counting chamber and Sperm 360 DNA Fragmentation, examination of the seminal fluid of two hundred and one males to look for signs of infertility. It was done by looking for aberrant sperm in the sample. During examinations of the two hundred and one patients for infertility, it was discovered that the rate of male infertility stands at 57.21%. The asthenozoospermic was 36.32%, oligozoospermic was 1.49%, and azoospermia was 8.96%. Oligosthenozoospermia was reported to have been 7.96% and polyzoospermia1.00%, respectively. DFI between fertile and infertile men was (11.02 vs. 37.88, p<0.001). Therefore, testing for sperm DNA fragmentation after semen analysis is an effective method for determining the cause of infertility. The prevalence rate of men with infertility was 57%, indicating that it was significantly higher than expected. As a result, appropriate treatment procedures must be carried out while managing male factor infertility.

Keywords: Sperm, infertility, sperm DNA fragmentation, infection, semen analysis.

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1. INTRODUCTION

World Health Organization (WHO) has defined infertility as a male or female reproductive system condition characterized by the inability to obtain pregnancy after 12 months or more of frequent unprotected sexual intercourse[1]. According to available data, infertility affects approximately 13% to 15% of all couples worldwide, with one in every five failing to conceive during the first year[2]. In India, infertility rates range from 3.7% in Himachal Pradesh, Uttar Pradesh, and Maharashtra to 5% in Andhra Pradesh and 15% in Kashmir[3]. Generally, the male factor contributes significantly to approximately 50% of all fertility issues[4]. It is noteworthy that in 20% to 30% of infertility instances, male infertility contributes 50% to fertility issues in couples. Male infertility is described as a male's inability to impregnate a fertile female after at least one year of unprotected intercourse. Africa and Central and Eastern Europe recorded the highest rates of male infertility, while rates in Central and Eastern Europe, Australia, and North America ranged from 8-12%, 9%, and 4.5-6%, respectively[5]. The accurate frequency of male infertility is unknown since it is not a notifiable disorder. Besides that, the treatment is usually paid privately and does not appear in insurance coverage records. In addition, male infertility is frequently addressed in clinical settings, and such information is seldom included in big clinical data sources[6]. A growing risk is the commonly publicized global trend of sperm count declines over the last few decades. In 1940, the average sperm count was 113 million/ml; by the 1990s, it had dropped to 66 million/mL[7]. Although the underlying role seems to be unknown, potential causes include prolonged contact with environmental toxicants and enhanced worldwide healthcare, which enables more men with poor well-being to play an active role in fertility practices. The disclosed decline in sperm quality could also be due to variations in procedures, conflicting research facility requirements, and differing measurement methodologies. Indian males' vital sperm characteristics have also deteriorated, according to studies[8]. These researches have proven that the region's sperm quality had deteriorated mostly because of nutritional, lifestyle, and environmental factors. Over 20 years (August 1990 to July 2005), a prospective and retrospective investigation of infertility cases in the Indian population revealed that the average prevalence rate of male infertility ranged from 8.97% to 14.63%[9]. Male infertility is generally triggered by male factors such as sperm deficiencies, genetic and congenital characteristics, anatomical abnormalities, endocrine disorders, immunological or functional anomalies, sexual aspects inconsistent with sexual contact, and chronic disease[10]. According to growing findings, sperm DNA fragmentation (SDF) plays an impartial and impressive function in male infertility and reproduction rate[11,12,13]. SDF is the alteration of the DNA structure or formation of adducts causing single or double strands of DNA breaks in the DNA and potentially adversely impacting fertility. SDF has received increased attention as a significant reason for male infertility over the last ten years and is currently being studied extensively. Sperm DNA damage occurs during spermatozoa production/maturation or transport through the male genital tract[14]. The integrity of sperm DNA is critical for precise genetic information transmission, and any sperm chromatin abnormality or DNA damage can result in male infertility[15].Infertile males quite often have elevated amounts of SDF in their sperm. According to a 2018 comprehensive study and meta-analysis that included about 4,000 men and 27 research, the mean score distinction in SDF rates among fertile and infertile men was 1.6%[16]. Elevated SDF levels have been linked to various conditions, including accessory gland infections, advanced paternal age, varicocele, chronic illness, medications, ionizing and non-ionizing radiation, heat stress, lifestyle, obesity, and occupational and environmental variables[17,18]. These conditions can induce SDF primarily by impairing spermatogenesis, inducing abortive apoptosis, or boosting the production of reactive oxygen species (ROS). Increased ROS is an important driver of SDF in live spermatozoa[19]. Although conclusive, human studies are underwhelming; sperm DNA damage has been linked to low reproductive success, impeded embryogenesis, an elevated incidence of miscarriage, rising mortality rates in offspring, and childhood leukemia[17,18,20-]. Semen analysis has always been the cornerstone of male fertility evaluation, but despite years of testing and improvement, it is still inexact, with low sensitivity and specificity[21].Although many assessments have been developed to evaluate sperm chromatin integrity and SDF, professional groups usually do not support routine SDF testing in semen analysis. The deoxynucleotidyl transferase-mediated dUTP nick end labeling assay (TUNEL), the sperm chromatin structure assay (SCSA), the single-cell gel electrophoresis assay (Comet), and, more recently, the sperm chromatin dispersion test is among the most frequently performed DNA integrity tests (SCDt). Various research using these methods to analyze sperm DNA integrity have identified an important link between sperm DNA damage and pregnancy complications in humans[22]. Nevertheless, the incidences of spermatozoa with DNA damage differ substantially, likely due to the testing methods for DNA integrity and the populations under study that are associated with clinical risk factors such as smoking and alcohol exposure. Smoking triggers poor sperm quality and sperm DNA/genetic damage[23,24], and alcohol consumption has also been linked to sperm DNA integrity[25,26].

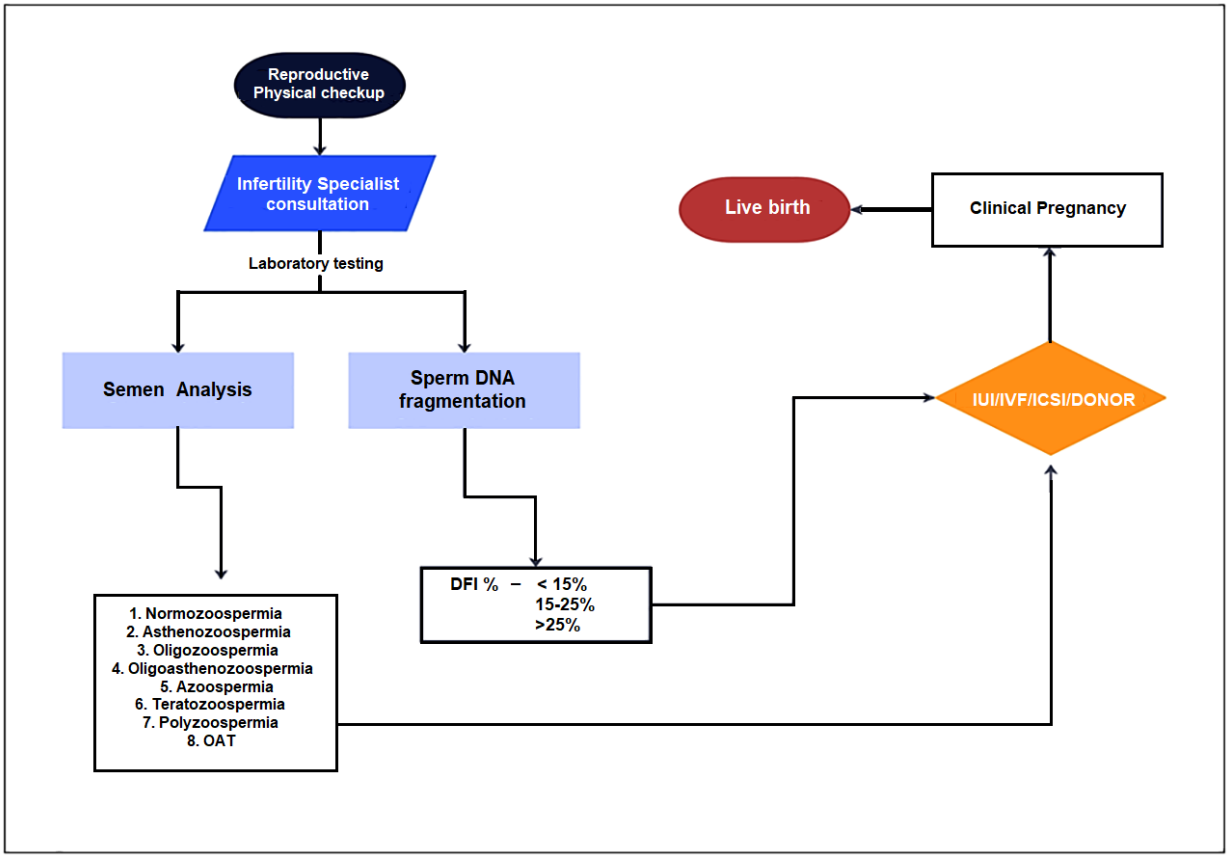

Fig 1: Flowchart of workflow for fertility treatment

Figure 1 illustrates the following process, beginning with the patient's first visit to the clinic or hospital and continuing through the fertility consultation, laboratory diagnosis, treatment plan, clinical pregnancy, and live birth. In this regard, we aim to report the first documented prevalence rate of male infertility in Meghalaya, India. Our objective is to evaluate semen samples of married men to investigate the cause of male infertility. The study will evaluate the different disorders in the semen and sperm with special emphasis on sperm DNA fragmentation in male patients attending a tertiary care hospital in Meghalaya, India, and compare the results obtained from conventional semen analysis with that obtained using sperm chromatin structure assay.

1.1. ETHICAL STATEMENT

It was granted Institutional Ethical Clearance (IEC) (No.18(A)/research etc/GDH/2020-21/9577) which is by the guidelines of the Declaration of Helsinki for biomedical research on human subjects. Furthermore, participants' consent was collected regarding the provision of anonymity of the participants, data storage, study conduct, and publication.

Inclusion Criteria

1. Participants with an age group of > 18 years
2. Only men were included
3. Participants should be married

Exclusion Criteria

1. Unmarried men
2. Female patients

2. MATERIALS AND METHODS

In the current study, 201 couples who visited the infertility clinic at the Department of Obstetrics and Gynecology, Ganesh Das Govt. M&CH Hospital, Shillong, Meghalaya, India. The study was carried out between July 2021 and July 2022. After a mandatory 2–7-day period of sexual abstinence, the infertile patients' semen samples were taken. Meckler's chamber was used for sperm counts estimation, and the smear diff fast stain method was used for the morphological investigation. The World Health Organization's recommendations were followed when examining the sperm. The infertile individuals were divided into three groups based on sperm concentration: normozoospermic, asthenozoospermia, teratozoospermia, oligo asthenozoospermia, oligoasthenoteratozoospermia, and azoospermia (no spermatozoa). SCD testing was used to determine the degree of sperm DNA fragmentation. A kit called Sperm 360 DNA Fragmentation was used to perform the test on the sperm (Sperm Processor Pvt. Ltd., Aurangabad, India). This kit is based on a technique initially updated by Chauhan KR et al. using 0.4 M dithiothreitol (DTT) and demonstrated by Fernandez JL et al.[27,28]. At 37°C, a portion of the semen sample is mixed with 1% low melting agarose. The mixture was pipetted into 60-L aliquots, spread out on slides, and covered with coverslips. At four °C, slides were allowed to settle. After that, reagent I (lysis solution) was applied to the slide for seven minutes. Next, reagent II (neutralizing and lysis solution 2) was applied, after which the slide was rinsed with distilled water. The slide was then gradually dehydrated in 70%, 90%, and 100% ethanol for two minutes. The microscope stain included in the kit was used to stain the slide. The slide was then looked at under a bright field microscope. Two hundred sperms were examined for halo size and dispersion pattern on each slide, looking for 1) nuclei with large DNA dispersion halos; 2) nuclei with medium-sized halos; 3) nuclei with small-sized halos; and 4) nuclei without a halo, as described by Fernandez JL et al.,[29]. The nuclei with a big to medium-sized halo were thought to have intact DNA

(dispersed nuclei). Fragmented DNA (non-dispersed nuclei) was defined as nuclei with a modest or no halo [Table-1]. Table 1 illustrates the normal reference value provided by the 2021 WHO guidelines for examining human semen[36]. The varying halo diameters are broken down here in Table 2. A large halo indicates normal sperm, but an absence of a halo indicates DNA fragmentation in the sperm. DFI was estimated as a percentage of the total number of spermatozoa counted, the percentage of spermatozoa having DNA fragments. Further categorization of the participants was done using DFI cut-off values of 25%, 15%-25%, and <15%[30].

Table 1:Normal values for semen parameters.

Semen parameters	WHO 2021 Normal Value
Motility	>= 42 %
Concentration	>= 16 Million/ml
Morphology	>= 4 %

3. STATISTICAL ANALYSIS

Statistical analysis was done using R-software to evaluate the data. To determine the significance of the study, a one-sample t-test and an unpaired two-sample t-test were used, confidence intervals were estimated, and the p-value was calculated. Any result less than or equal to 0.05 was regarded as statistically significant, and the significance level was set at α=0.05.

4. RESULTS

Two hundred and one (201) male semen specimens were taken and 115 (57.21%) (Table 4) of those were found to be infertile due to poor seminal fluid sperm density, motility, and morphology.

Table 3: Different pathological classifications saw in the total study

Semen analysis report	Number of patients	Total percentage
Normozoospermia	86	42.79
Asthenozoospermia	73	36.32
Azoospermia	18	8.96
Oligoasthenozoospermia	16	7.96
Oligoasthenoteratozoospermia (OAT)	3	1.49
Oligozoospermia	3	1.49
Polyzoospermia	2	1.00
Teratozoospermia	0	0.00

Table 3 shows the different pathological classifications of male infertility in Meghalaya, India. Asthenozoospermia (low motility) were the most prevalent of all groups except Normozoospermia (normal functioning sperms), which explains the reasons for the inability of many male patients to provide offspring for their partner. Azoospermia (absence of sperm) is the second most prevalent, followed by Oligoasthenozoospermia, Oligoasthenoteratozoospermia (OAT), Oligozoospermia, and Polyzoospermia, respectively.

Table 4: Male infertility rate in different regions/states

Different regions/states of India	Male Infertility (%)
Ahmedabad[31]	76
Delhi[32]	84
Jamnagar[33]	45
Bengaluru[34]	35
Kolar[35]	42
Wardha[36]	65
Present study	**57.21**

As shown in Table 4, the current study found a male infertility rate of 57.21% when compared with India's various states and regions. It is greater than Jamnagar, Bengaluru, and Kolar but lower than Delhi, Ahmedabad, and Wardha. It explains why Meghalaya, as a non-industrial state, has a lower rate, despite the rate rapidly increasing as time passes.

Table 5: Comparison of various parameters between the fertile and infertile groups.

Parameters	The mean of men of an infertile couple (N=110)	Mean of men with proven fertility (N=91)	p-value
Sperm motility	23.83	59.06	< 2.2e-16*
DNA fragmentation index %	37.88	11.02	< 2.2e-16*

To obtain a p-value, an unpaired two-sample t-test was used. *p-value <0.05 was significant. As seen in Table 5, there is a significant difference between the motility of infertile men and that of fertile men (23.83 vs. 59.06), which explains why

infertile men will have a lower level of motility in comparison to men with normal fertility. In addition, the DFI index% demonstrates a significant significance level between infertile and fertile men (37.88 vs. 11.02), which adds to the fact that infertile men have a higher DFI index than men who have proven fertility. It also demonstrates that the level of sperm motility is directly proportional to the DFI index; consequently, men with low motility are more likely to have higher levels of sperm DNA fragmentation than fertile men, which demonstrates their inability to fertilize oocytes during the fertilization process.

5. DISCUSSION

Since it affects 15% of couples of reproductive age, infertility is one of the major public health concerns[37]. The male factor is present between 40%- 50% of cases of infertility. Semen analysis is a commonly used technique to evaluate male reproductive potential. According to Macleod and Gold's 1951 recommendation, men should be regarded as fertile if their sperm counts are above 20 million per milliliter or their total count is above 100 million[38].In the current investigation, sperm DFI values were lower than 15% in 42.79% of normozoospermic cases and higher than 15% in 36.32% of asthenozoospermic cases (Table 3). It indicates that asthenozoospermic patients were considerably more likely than normozoospermic cases to have high sperm DFI values. There were no statistically significant variations in the sperm DFI values between infertile patients with normal or abnormal semen characteristics, according to Fernandez JL et al. (32.1±20.4 vs. 38.7±16.3, $p>0.05$)[27]. When using the SCD test, Fernandez JL et al. discovered a statistically significant difference between the sperm DFI values of healthy sperm donors and infertility patients (16.7±9.9 vs. 35.4±18.3, $p<0.05$)[27]. In their study, Wiweko B and Utami P discovered a significant difference in DFI between healthy fertile males and infertile men (19.9% vs. 29.9%, $p<0.001$)[39]. Proteins known as protamine and Transitional Protein (TP), both of which can be found in the nucleus of sperm, are the ones that are accountable for the compacting of sperm DNA while still preserving its integrity. During spermatogenesis, the sperm nuclear protein plays an essential function in maintaining the integrity of the compact sperm DNA[40-42]. The protamination process offers several distinct benefits. (a) DNA condensation results in a lighter nucleus, which makes it easier for sperm to ascend into the female genital canal. (b) improved DNA stabilization against free radicals created in seminal plasma resulting from sperm motility and metabolism. (c) the somatic epigenetic genes are removed from the sperm nucleus, which allows for free reprogramming by the oocyte following syngamy (d) a checkpoint in the process of spermiogenesis (defects in protamination can act as a checkpoint for the onset of apoptotic pathways) (e) activation of the oocyte following fertilisation[43,44]The lack of the amino acid protamine is the cause of DNA damage in sperm. In our study, similar results were obtained that support other existing studies in which DFI between healthy fertile males and men who were infertile (11.02vs.37.88, $p<0.001$) (Table 5). There is a link between the sperm DFI value and the clinical pregnancy outcome in several investigations. There is an important link between sperm DFI value and pregnancy rates with IUI and IVF, as indicated by meta-analysis and systemic review by Zini A, Osman A, et al., and Agarwal A et al.[45-47]. The odds ratio (OR) was 9.9, and the significance level was less than 0.001. Furthermore, Zini A. et al. found that the DFI value of the sperm was related to the number of miscarriages that occurred during in vitro fertilization and intracytoplasmic sperm injection (combined OR=2.48, p0.0001)[48]. Our study showed that semen analysis diagnostic tests alone could not diagnose male infertility. Rather it has to be in conjunction with another advanced sperm testing such as Sperm DNA fragmentation. Most medical professionals are not much aware of the importance of this diagnostic test.

6. LIMITATIONS

The following drawbacks of the current study design exist: 1) A small sample size was used in the study; 2) Participants were chosen from a single institute, and as a result, they might not be fully representative of all patients with sperm DNA fragmentation in the general Meghalayan population. In many parts of India, numerous additional environmental elements may contribute to sperm DNA damage.

7. CONCLUSION

When compared, a substantial difference in the sperm DFI of the men of infertile couples was found. Testing for sperm DNA fragmentation is an effective method for determining the cause of infertility. According to the current study, the rate of male factor infertility is alarmingly rising in the Indian state of Meghalaya. DNA fragmentation increases as paternal age rise. As a result, it is strongly recommended that sperm quality be given special consideration when evaluating the overall results of semen analysis and that routine sperm DNA fragmentation be a crucial component of a formal investigation in infertility clinics to enhance IUI and IVF successful outcomes. Male infertility can be efficiently treated, and more individuals can be cured with lifestyle changes, antioxidant supplementation, and antibiotic therapy in bacterial urogenital infections.

8. ACKNOWLEDGEMENT

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9. AUTHORS CONTRIBUTION STATEMENT

BCH created the initial concept and every element of its execution. The study's conception and design, as well as the drafting of the manuscript, were done by BCH, PP, and KSM. PP and KSM approved the final version after critically examining the original draft. REK and BCH carried out the tabulation and statistical analysis of the data. Finally, BCH and REK finalized the results and conclusions. The final draft has been carefully examined and approved by all authors.

10. SOURCE OF FUNDING

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11. CONFLICT OF INTEREST

Conflict of interest declared none.

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