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RESEARCH ARTICLE

EVALUATION OF SEMEN SAMPLE PARAMETERS IN MALE INFERTILE SUBJECTS AND ITS CORRELATION WITHY CHROMOSOME MICRODELETION IN POPULATION OF DODA CITY, JAMMU AND KASHMIR

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Abstract

Introduction: More than half of reproductive difficulties cannot be attributed to female characteristics. A number of illnesses and conditions that may have an impact on male fertility have been found via recent research. A number of underlying diseases and health issues, as well as environmental variables, may contribute to male infertility. For a sexually active couple, infertility is diagnosed after a year of unsuccessful attempting to conceive. Infertility affects around 19% of couples who have trouble conceiving. Any and all racial and ethnic groups may feel at home within these parameters.

Materials and Methods: The study was conducted in 100 infertile patients and 50 fertile subjects were use as controls. The sample will be obtained by masturbation and ejaculated into a clean and proper labeled, wide-mouthed container made of glass or plastic according to the rules laid down by WHO.

Result and Conclusion: The sperm count in infertile and fertile subjects were 15.8 ± 2.85 and 56.57 ± 8.47 respectively, Motility 37.6±1.8and 54.4 ±1.8, volume 2±0.8 and 2.6±0.1, viability 53.7±1.4 and 66±1.2. Out of 100 infertile patient's 5 percent subjects had Y chromosomal microdeletions (5 samples of 100).

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Introduction:-

Spermatogenesis is a complex biological process taking place in the seminiferous tubulesof the testes where the transformation of spermatogonial cells into spermatozoa takes place. Endodermal cells in the human yolk sac differentiate into Primordial Germ Cells (PGC)[1]. These signals go from the brain to the gonads through the dorsal mesentery. Cells along the gonadal ridge have the potential to differentiate into ovaries in females and testes in males. The sex-determining region Y (SRY) gene is first expressed in the pre-Sertoli cells of developing male gonads. The Sertoli cell and the Leydig cell are two types of diploid cells that help produce sperm. Sertoli cells are located deep inside the seminiferous tubules, while Leydig cells are located on the periphery and in the interstices. Sertoli cells secretes Anti-Müllerian hormone, which prevents the development of Müllerian ducts in the embryo. Leydig cells generate the hormone testosterone, which promotes the development of both the interior and exterior male genitalia [2]. PGCs guide the differentiation of progenitor cells into type A spermatogonia in the seminiferous tubules. Type A spermatogonia may either continue dividing to make more of the same or they can differentiate to make a new kind of spermatogonia. Meiosis-competent spermatocytes are only produced by type B spermatogonia. Primitive spermatocytes undergo mitotic cell division over the period of 16 days. Through two rounds of specialised cell divisions called meiosis I and meiosis II, the number of chromosomes is decreased from a diploid to a haploid

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complement. DNA replication occurs during meiosis I in spermatogonia to generate new spermatocytes[3]. Using the haploid cells that have survived meiosis, sperm are formed by a process called spermiogenesis [4]. The synthesis of the acrosomalcap, flagella, extra cytoplasm, and chromatin occurs in a process known as spermiogenesis, the process responsible for the production of mature spermatozoa.

Clinical diagnosis depends on semen analysis for the assessment of male infertility and also for evaluating efficacy of male fertility regulating agents. However, sperm count and sperm motility are the first important prediction of fertility potential [5].

Male infertility is a reproductive well-being problem that affects the couples in the humanpopulation. Infertility is defined as a failure to conceive in a couple trying to reproduce for aperiod of two years without conception. Approximately 15 percent of couples are infertile, andamong these couples, male factor infertility accounts for approximately 50 percent of causes [6].It is a multifactorial syndrome which causes a wide variety of disorders. In more than half ofinfertile men, the cause of this problem is unknown and could be congenital or acquired.Infertility in men can be diagnosed initially by semen analysis. Spermograms of infertile menmay reveal many abnormal conditions, which include azoospermia, oligozoospermia,teratozoospermia, asthenozoospermia, necrospermia and pyospermia. The current estimate is thatabout 30 percent of men seeking help at the infertility clinic are found to have oligozoospermiaor azoospermia of unknown aetiology. Therefore, there is a need to find out the cause atpathological, biochemical and genetic level [7].

Materials And Method: -

The study was conducted in 150 subjects. (100 test and 50 controls), aged between 23-30 years in Government Medical College and Associated Hospital, Doda city, Jammu and Kashmir and GK labs, Srinagar for molecular testing. The Semen sample was collected after proper consent as per the guidelines laid down by World Health Organization (WHO).

Semen sample collection

The sample was obtained by masturbation and ejaculated into a clean and proper labeled, wide-mouthed container made of glass or plastic. The specimen container was placed on the bench while the semen liquefies. The specimen container was kept at ambient temperature, between 20 °C and 37 °C, toavoid large changes in temperature that may affect the spermatozoa.

Sperm count:

The number of spermatozoa in the ejaculate was calculated from the concentration of spermatozoa and measured. 10-15 µL of semen sample was taken on a neubarhaemocytometer and a cover slip was placed over it. The sample was allowed to settle down for about 5 mins. The number of spermatozoa in the central square ofNeubauer counting chamber was counted. The chamber has 25 large squares each of which is dividing lines of the square then only those lying on the upper, left line were counted.

Motility:

Sperm motility within semen was assessed right after liquefaction of thesample, preferably at 30 minutes, but in any case, within 1 hour, following ejaculation, to limit the deleterious effects of dehydration, pH or changes in temperature on motility. Sperm were collected, filtered, and resuspended in sperm nutrition solutions with pH ranging from 5.2 to 8.2 for 15, 30, 60, 90, and 120 minutes to allow for development. Sperm motility was measured using a computer software called computer-assisted semen analysis (CASA: WLJY-9000; Weili New Century Technology Development Co., Ltd.; Beijing, China).

Semen pH:

The sample was mixedwell and a drop of the sample was spreaded evenly on the pH paper. We compared the colour with the calibration strip to read the pH of the sample.

Semen volume:

Volume of the ejaculate will be measured to the nearest 0.1 ml in a graduated centrifuge tube. The volume of the sample would be categorized as normal range in between 1.5 ml to 4.5 ml.

Molecular Analysis:

Genetic testing for Y chromosomal deletions:

Multiplex PCR reactions A and B were used to identify microdeletions in AZF areas. Multiplex PCR is indicated for the diagnosis of Y chromosome microdeletions in the AZF region, and the following primer sets were used: sY14, ZFX/ZFY as male and mixed-gender controls; sY84, sY86 for AZFa; sY127, sY134 for AZFb; and sY254, sY255 for AZFc deletions. Y chromosomal microdeletions were analysed by using a commercial kit, Y Chromosome Deletion Detection System, Version 2.0.

Results:-

Sperm count:

Based on the present study, 4 different infertile groups were demonstrated as depicted in table 1.

S.no	Infertile subgroups	n = 100	Percentage
1	Aspermia	12	12%
2	Azoospermia	49	49%
3	Oligozoospermia	16	16%
4	Teratozoospermia	26	26%

Table 2:- Comparison of semen parameters between fertile and infertile subjects.

Semen parameters	Test (n=100)	Control (n=50)	P value
Sperm count	15.8 ± 2.85	56.57 ± 8.47	<0.0001
Motility	37.6±1.8	54.4 ±1.8	<0.0001
Volume	2±0.8	2.6±0.1	<0.0001
Viability	53.7±1.4	66±1.2	<0.0001

pH: WHO criteria for normal pH ranges from 7.2 -7.8.

Table 3:- Shows the observed value of semen pH in 100 patients.

pH	Total subjects=100
>7.8 (alkaline)	69 %
7.2-7.8 (normal)	21%
<7.2 (acidic)	10%

Y chromosome microdeletions:

Among the men who were tested for idiopathic infertility, 5 percent had Y chromosomal microdeletions (5 samples of 100). Five microdeletions were identified in the AZFc area in three of the instances, and all three AZF regions were deleted in two of the cases (AZFa+b+c deletion). Infertile males with the most severe forms of infertility, azoospermia and oligozoospermia, were the only ones in whom microdeletions were discovered. The number of spermatozoa in spermatograms varies in infertile men with deletion in the AZFc region of the long arm of the Y chromosome (three instances). In one case, no spermatozoa were discovered (azoospermia), whereas in the other two cases, 1-5 immobile spermatozoa were found (severe oligozoospermia). As can be seen in the spermatogram data, azoospermia is the phenotype in the two instances of people who have a deletion of the whole AZF region (AZFa+b+c deletion). In both cases, cytogenetic examination showed an aberrant karyotype due to a deletion in the long arm of the Y chromosome (46, X, del(Y) (q). All three AZF deletions were found in a single individual. A clinical evaluation revealed that this person had central type obesity and a short neck. DNA samples from patients with Y-chromosome microdeletions were analysed many times using a commercial kit so that researchers could compare the results (Promega 2.0). Data of Y chromosome microdeletion analysis in five patients with AZF region microdeletions.

S.no	Deleted region	Spermatozoa in spermogramm	Clinical diagnosis	Karyotype	Phenotypic data
1	AZFc	0	Azoospermia	46, XY	Android adiposity, gynaecomastia, brachydactyly
2	AZFc	5 (motionless	Severe	46, XY	Epidemic

		spermatozoa in spermogramm	oligozoospermia		parotitis in childhood, in testis biopsy only spermatids found
3	AZFc	1-2 immobile spermatozoa in spermogramm	Severe oligozoospermia	46, XY	Epidemic parotitis in childhood
4	AZFa AZFb AZFc	0	Azoospermia	46, X del (Y)(q)	Android adiposity. Gynaecomastia, small testis in right side
5	AZFa AZFb AZFc	0	Azoospermia	46, X del (Y)(q)	No data

Discussion:-

After the Klinefelter syndrome, microdeletions of the Y chromosome are the most common hereditary cause of spermatogenetic failure in males. Many molecular genetics labs across the globe use techniques to routinely detect Y chromosomal microdeletions. One of the goals of this research was to legitimise and popularise this fine-tuned approach of investigation in Jammu and Kashmir. Cytogenetic (chromosome staining and microscopic examination) procedures, clinical anamnesis, or sperm analysis cannot be used to identify Y chromosome microdeletions. Therefore, polymerase chain reaction (PCR) and other molecular diagnostic procedures are required. What does it mean when microdeletions on the Y chromosome are spotted? Because of this method's introduction, we were able to ascertain that the prevalence of Y chromosomal microdeletions in men with idiopathic infertility is around 5%, which is much higher than the previous estimate of 1%. Furthermore, the validated approach may be used as a standard screening approach for cases with idiopathic male infertility. This means that for certain individuals with Y chromosome microdeletions, identification of microdeletions may confirm a diagnosis of infertility, determine whether or not in vitro fertilisation is a viable option, and provide valuable information for genetic counselling.

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