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Genetics

CYTOGENETICS AND MOLECULAR STUDIES ON OLIGO/AZOOSPERMIA

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ABSTRACT Infertility and problems of impaired fecundity have been a concern through ages and is also a significant clinical problem today, which affects 8–12% of couples worldwide. Of all infertility cases, approximately 40–50% is due to "male factor" infertility and as many as 2% of all men will exhibit suboptimal sperm parameters. It may be one or a combination of low sperm concentration, poor sperm motility, or abnormal morphology. Hence the present study aimed to evaluate the cytogenetic and molecular studies on oligo/azoospermic subjects. Here 53 study subjects and twenty seven healthy control subjects were selected for cytogenetic and molecular studies. DNA damage was evaluated using Cytokinesis block micronuclei assay. Observations from the study analyze that study subjects were showed highest mean CBMN frequency than controls. Subjects with risk factors showed increased micronuclei frequency. Avoid modern lifestyle with regular exercise may to escape from male infertility.

KEYWORDS : Infertility, Oligospermia, Azoospermia, karyotype and Cytokinesis block micronuclei assay

INTRODUCTION:

Male infertility, which refers to a male's inability to cause pregnancy in a fertile female, is mostly caused by spermatogenic failure. Problems during spermatogenesis contribute to a lower or absent production of spermatozoa (Massart et al., 2012). Oligospermia is defined as a low sperm concentration (<20 million sperm/mL) in the ejaculate (World Health Organization, 1999). Azoospermia defined as complete absence of sperm from the ejaculation, is present in less than 1% of all men and in 10 to 15% of infertile men (Constantin-Cristian Vaduva et al., 2016).

Major etiology behind Oligo/Azoospermy is testicular trauma (Shaul DB et al., 1997), vasectomy, diabetes mellitus (Sharpe RM, 2010), varicocele (Antoniassi et al., 2014) and obesity (Shukla et al., 2014). The application of physical agents (heat, electromagnetic energy, or ultrasound) and environmental toxicants (cadmium and mercury) can suppress spermatogenesis (Thonneua et al., 1998). Neurogenic reproductive dysfunction in men with spinal cord injury (SCI), ejaculatory failure and abnormal semen characteristics were observed in infertile man (Prasad Patki et al., 2008).

Several studies suggested that Reactive Oxygen Species (ROS) attacks the integrity of DNA in the sperm nucleus by causing nucleotide modifications, DNA strand breaks, and chromatin cross-linking (Benchaib et al., 2003). Chromosomal abnormalities account for approximately 6% of infertility in men, and the prevalence increases to 15% among men with azoospermia (Brugh et al., 2003). 47, XXY is the most common sex chromosome aneuploidy in human males with two or more X chromosomes, occurring in approximately 1:500 newborn males (Oates, 2003).

Chromosomal translocations may cause the loss of genetic material at the breakpoints and could result in testicular failure (Martin, 2008). Robertsonian translocation occurs when two acrocentric chromosomes fuse with an incidence of approximately 1:1000 and may affect spermatogenic

process (Antonelli et al., 2000). Y chromosome microdeletions are found in 10%–15% of men with nonobstructive azoospermia or severe oligospermia (Simoni et al., 1997).

The genetic instabilities due to the Oligo/Azoospermy can be diagnosed by the extent of DNA damage and the micronuclei frequency present in the lymphocytic cells. The development of the cytokinesis block micronuclei assay (CBMN) is a reliable and precise method for assessing chromosome damage. Chromosomal abnormalities were determined with male mitotic karyotype analysis from peripheral blood through chromosome banding techniques. An estimated 15% of the cases of male infertility are caused by gene and chromosome anomalies (Maria et al., 2016).

Defects in reproductive health of a human will cause future health problems and it also predicts future cardiovascular diseases. So it is important to study cytogenetic and molecular studies on infertility. Hence the present study was undertaken to quantify the extent of DNA damage by cytokinesis block micronuclei (CBMN) assay along with evaluating chromosomal abnormalities in infertile subjects using lymphocyte culture.

MATERIALS AND METHODS:

Fifty three subjects affected with varying degrees of Oligo/Azoospermy were selected for this study and twenty seven healthy subjects were also selected as control for this study. The samples were referred from various infertility hospitals of kerala to Genetika, Centre for Advanced Genetic Studies, Thiruvanathapuram, Kerala. Demographic, physiologic and lifestyle characteristics were recorded using proforma.

Four ml of venous blood was collected aseptically from all the subjects by venipuncture after overnight fasting and transferred into the vacutainer containing sodium heparin to perform lymphocyte culture and CBMN assay. 5-6 drops of heparinized blood was added to a culture tube containing 10 ml of RPMI 1640 media supplemented with 15% of fetal bovine serum and 10µg/mL phytohaemagglutinin.

Cytochalasin B was added to the cultures at a final concentration of 4.5µg/mL after 44th hour. Cells were harvested after 72 hr incubation, and they were treated with a hypotonic KCl solution (0.075M KCl) for 10 min and fixed in fresh fixative solution (methanol: acetic acid, 3:1). The cells were dropped onto slides and the slides were air dried and stained with 10% Giemsa. Micronucleated cells were analyzed under a microscope at 100X magnification. The number of micronuclei is not less than 1000 binucleated cells were scored and the distribution of micronuclei among binucleated cells was recorded.

OBSERVATIONS AND RESULTS:

53 infertile study subjects with age between 27 to 52 years. Their average age was 35.22 years. 27 control subjects were belongs to 30 to 41 years and their average age was 36.37 years. The birth order of the subjects was from 1 to 8. Majority of subjects were belonged to <3 birth order. Majority of the subjects were residing in urban area. Most of the subjects have sedentary type work. Average weight of study subjects were 75.89 kg. On 53 subjects, 27 have diabetes and 18 have hypertension. 28.3% of subjects with family history of infertility.

Lifestyle characters such as smoking, chewing and drinking were also observed in study subjects. Among these infertile subjects, 41 subjects reported with oligospermia and 12 subjects were reported azoospermia.

Distribution Of Mean Cbmn Frequency According To Subjects
Table 1:

Subjects	Number	Karyotype		Mean CBMN frequency
		Normal	Abnormal	
Study subjects	53	44	9	12.90
Control subjects	27	27	0	10.26

Table 1, tabulate the comparison of mean CBMN frequency according study and control subject. 53 study subjects were showed 12.90 as mean CBMN frequency and 9 subjects with abnormal karyotype. 27 control subjects have mean CBMN frequency of 10.26.

Distribution Of Mean Cbmn Frequency According To Demographic Characteristics Of Subjects
Table 2:

Demographic characters of the subjects were given in table 2.

Category	Variables	Number (%)	Karyotype		Mean CBMN frequency
			Normal	Abnormal	
Age (Years)	<30	9 (16.6)	7	2	11.88
	≥30	44 (83)	37	7	13.11
Birth order	≤3	34 (64.1)	30	4	12.79
	>3	19 (35.8)	14	5	13.1
BMI (Kg/m^2)	<25	10 (18.8)	9	1	11.96
	25-30	33 (62.2)	28	5	13.02
	>30	10 (18.8)	7	3	13.45
Religion	Hindu	32 (60.3)	28	4	12.99
	Christian	10 (18.8)	7	3	12.88
	Muslim	11 (20.7)	9	2	12.67
Residence	Coastal	6 (11.3)	4	2	12.79
	Rural	29 (54.7)	25	4	12.88
	Urban	18 (33.9)	15	3	12.97
Education achievement	Primary	6 (11.32)	5	1	13.17
	Secondary	8 (15.09)	8	0	12.43
	Higher secondary	16 (30.1)	13	3	13.17
	Graduates/PG	23 (43.3)	18	5	12.8
Occupation	Sedentary	29 (54.7)	24	5	13
	Non-sedentary	24 (45.2)	20	4	12.8
Parental consanguinity	Yes	16 (30.1)	10	6	13.54
	No	37 (69.8)	34	3	12.63

Age of the subjects were grouped into <30 and >30 years. Advanced age of the subjects was showed highest mean CBMN frequency. On their BMI, subjects were grouped into normal, overweight and obese. Highest number of abnormal karyotype and mean CBMN frequency was shown by subjects with overweight and obesity. Majority of the subjects were Hindus and Christian's posses increased mean CBMN frequency.

Subjects residing in urban area were showed highest mean CBMN frequency. Most of the subjects have sedentary type of occupation and they having highest mean CBMN frequency of 13. 16 subjects reported with parental consanguinity, of these 6 having abnormal karyotype.

Clinical and lifestyle characters of the subjects were given in table 3. Subjects with clinical characters such as H/o diabetes, H/o hypertension, H/o dyslipedimia and family H/o infertility were showed highest mean CBMN frequency. 26 subjects were smokers, 19 subjects were chewers and 24 having habit of alcohol consumption. All these subjects have genetic instabilities.

Distribution Of Mean Cbmn Frequency According To Semen Analysis
Table 3:

Variables	Category	Number	Karyotype		Mean CBMN frequency
			Normal	Abnormal	
Semen analysis	Azoospermy	12 (22.6)	6	6	13.8
	Oligospermy	41 (77.3)	38	3	12.64

According to semen analysis, 12 azoospermic subjects were showed 6 abnormal karyotype and showed mean CBMN frequency of 13.8. 41 oligospemic subjects have mean CBMN frequency of 12.64, of these 3 of them showed abnormal chromosome pattern.

DISCUSSION:

Hassan, (Hassan & Killick, 2003) reported that evidence for the decline in men's fertility with increasing age. Plas et al., (2000) reported that increase of spermatozoa structural aberrations with age. Isiah et al., (2011) observed that aging has a significant impact on male sexual function, sperm parameters, and fertility. In the present study reported that mean CBMN frequency was higher in subjects with age ≥30 years (13.11). Asare et al., (2016) reported that sperm motility was significantly lower in smokers compared with nonsmokers. The result from this study was severe DNA damage found in smokers than non smokers.

Rosita et al., (2013) reported that alcohol consumption seems to alter sperm parameters and testicular pathology. As far as sperm parameters, the more frequently abnormality reported is the higher percentage of morphologically abnormal spermatozoa. In addition, decrease in the seminal fluid volume and increased seminal fluid leukocyte concentration has also been reported. The present study conducted in 24 subjects the mean CBMN frequency of subjects with habit of alcohol consumption (13.3) was higher than that of the subjects without the habit of alcohol consumption (12.57).

Bushardt et al., (2008) reported in their study was all diabetic men are infertile and there are conflicting results concerning the effect of DM in sperm parameters but, even when conventional sperm parameters do not differ, the glucose transport mechanisms are expected to be altered. Glucose availability and/or insulin dysfunction induced by DM are expected to induce important metabolic adaptations in testicular cells, besides changes in oxidative stress that are often reflected in mitochondrial and nuclear DNA fragmentation. In the present study mean CBMN frequency was carried out in the 27 diabetic subjects was 13.04 and it was higher than that of the subjects without diabetes (12.76).

A recent report by Rera et al., (1995) showed that specific epididymal sperm membrane coating protein production decreased when epididymal cells were subjected to X-ray exposure indicating a molecular basis of effect of radiations in the epididymis. In the present study, subjects with H/o X –ray exposure shows high mean CBMN frequency (13.57) than that of subjects without X –ray exposure (12.66).

CONCLUSION:

From the present study it can be concluded that mean CBMN frequency was dependent variable with age, parental consanguinity, sedentary occupation, family H/o infertility/subfertility, semen analysis and hypokinetic diseases etc. The mean CBMN frequency showed an association with various demographic, clinical, biochemical, physiological and lifestyle characteristics of subjects. Avoid modern lifestyle such as sedentary occupation, habits of alcohol consumption, smoking and chewing, reduce body weight through regular exercise and reduce fast food consumption, precautions from the exposure of radiation and temperature in work place. It will be the most convenient method to escape from oligo/azoospermia.

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