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Genetics

CYTOGENETIC AND MOLECULAR STUDIES ON MALE INFERTILITY

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ABSTRACT Male infertility is responsible for nearly half of the infertility and affects one man in 20 in general population. Infertility is seen in about 15% of the married couples and about 30-50% of the cases are due to male factors. A large majority of infertile men have associated genetic disorders that range from chromosomal aneuploidy or structural rearrangements to mutations or microdeletions. Precise determination of full range of factors contributing to infertility needs detailed evaluation. Hence the present study was undertaken to evaluate the cytogenetic and molecular aspects of male infertility. Thirty two study subjects with a clinical diagnosis of male infertility were selected as study subjects and twenty subjects without any chronic illness were selected as controls. In this study, Cytokinesis Block Micronuclei (CBMN) assay for quantifying the DNA damage and Karyotyping for chromosome aberrations were performed in each subject. The present study revealed that male infertile subjects showed increased mean CBMN frequency and chromosomal aberrations than healthy controls. Avoid modern lifestyles such as sedentary occupation, habit of alcohol consumption etc and taking precaution about environmental factors such as radiation exposure and temperature at workplace will help to minimize the risk for male infertility. The ideal marriage age, absence of addictions, timely medical assistance can help couples to become pregnant successfully.

KEYWORDS : Male Infertility, Microdeletions, Cytokinesis Block Micronuclei Assay, Karyotype And Dna Damage

INTRODUCTION:

Male infertility means the inability of male to achieve pregnancy in a fertile female. Male infertility is responsible for nearly half of the infertility (Mac Leod's, 1951; Zhang et al, 2009) and affects one man in 20 in the general population (McLachlan and Kretser, 2001). Male infertility commonly due to deficiencies in the semen and semen quality is used as a surrogate measure of male fecundity (Cooper T G et al, 2010). Male infertility can either be primary or secondary; primary male infertility is when the man has never achieved a pregnancy in woman, while secondary male infertility is when a man has achieved a pregnancy in woman irrespective of the outcome of the pregnancy. Men with secondary infertility, in general, have better chance of future fertility (Rowe PJ et al, 2000). A significant proportion of infertile males are affected either by oligozoospermia (reduced sperm production) or azoospermia (lack of any sperm in the ejaculate).

The main sign of male infertility is the inability to conceive a child. There may be no other obvious signs or symptoms. In some cases, however, an underlying problem such as an inherited disorder, hormonal imbalance, dilated veins around the testicle, or a condition that blocks the passage of sperm causes signs and symptoms (Mayo Foundation for Medical Education and Research, 2018).

Infertility is seen in about 15% of the married couples and about 30-50% of the cases are due to male factors. A large majority of infertile men have associated genetic disorders that range from chromosomal aneuploidy or structural rearrangements to mutations or microdeletions. Precise determination of full range of factors contributing to infertility needs detailed evaluation. Hence the present study was undertaken to evaluate the cytogenetic and molecular aspects of male infertility.

MATERIALS AND METHODS:

Thirty two study subjects with a clinical diagnosis of male infertility referred from various infertility centers of Kerala to Genetika, Centre for Advanced Genetic studies, Trivandrum were selected for this study. Twenty subjects without any chronic illness were also selected as control for this study. Detailed demographic, physiological, life style and biochemical characteristics of the subjects were recorded using proforma.

8 ml of venous blood was collected aseptically from all the subjects by venipuncture after overnight fasting. 4 ml of blood was transferred into the vacutainer containing sodium heparin to perform Karyotyping and CBMN assay. Remaining 4 ml of blood was transferred into plane tubes for biochemical analyasis such as Total Cholesterol, Fasting Blood Sugar and Urea.

Cytokinesis block micronuclei Assay (CBMN) described by Michael Fenech 1995 was performed by adding Cytochalasin B for quantifying the extent of somatic DNA damages. The frequency of micronuclei among 1000 binucleated cells were enumerated, recorded and analysed. Peripheral Blood Lymphocyte Culture was performed to analyse chromosome aberrations. Chromosomes were GTG banded according to Seabright in 1971. Karyotypes were prepared according to ISCN (International System for human Cytogenetic Nomenclature, 1995).

OBSERVATIONS AND RESULTS:

The distribution of mean CBMN frequency of the control subjects were 11.21 and study subjects were 13.05. The mean CBMN frequency of the study subjects was greater than the control subjects. Out of 32 study subjects 7 of them showed abnormal karyotype.

The demographic characters include age of husbands, birth order, residence, occupation and duration of married life. The age of the study subjects ranges from 29 to 50 years with a mean of 35.9 years and the control subjects ranges from 30 to 41 years with a mean age of 36.1 years. The birth order of study subjects ranged from 1-9. Regarding the residence, majority of subjects were in the rural area followed by urban and coastal area. Among 32 study subjects 18 subjects had sedentary occupation and 14 had non-sedentary occupation. Subjects had duration of married life ranged from 2-22 years.

Physiological characters include family history of infertility or subfertility and family history of cancer among first and second degree relatives. Among 32 study subjects 29 subjects had family history of infertility or subfertility. 28 subjects showed family history of cancer among first and second degree relatives. In the case of semen analysis, 5 subjects were azoospermic, 13 subjects were oligozoospermic and 14 were normozoospermic subjects. Among the 32 subjects 29 subjects showed history of X-ray exposure. Parental consanguinity was showed by 5 subjects. Study subjects who had habit of smoking, chewing and alcohol consumption were 10, 4 and 7 subjects respectively. The biochemical investigations revealed that total cholesterol >200 mg/dl was reported in 28 subjects. Subjects having

FBS value >110 mg/dl was showed by 26 study subjects. 30 study subjects showed urea level >15 mg/dl.

Distribution Of Mean Cbmn Frequency And Karyotype According To Demographic Characteristics Of Study Subjects
Table 1:

Variables	Category	Number (%)	Mean CBMN frequency	Normal karyotype	Abnormal karyotype
Age of Husbands (Years)	29-34	13 (40.62)	12.28	12 (92.30%)	1 (7.69%)
	35-40	12 (37.5)	13.58	7 (58.33%)	5 (41.66%)
	41-46	3 (9.37)	13.86	1 (33.33%)	2 (66.66%)
	47-52	4 (12.5)	13.96	1 (25%)	3 (75%)
Birth order	1-3	20 (62.5)	12.02	15 (75%)	5 (25%)
	4-6	8 (25)	12.92	6 (75%)	2 (25%)
	7-9	4 (12.5)	13.74	3 (75%)	1 (25%)
Residence	Costal	3 (9.37)	12.73	2 (66.66%)	1 (33.33%)
	Rural	19 (59.37)	12.15	15 (78.94)	4 (21.05%)
	Urban	10 (31.25)	13.67	3 (30%)	7 (70%)
Occupation	Non-sedentary	14 (43.75)	12.52	10 (71.42%)	4 (28.57%)
	Sedentary	18 (56.25)	13.69	7 (38.88%)	11 (61.11%)
Duration of married life	2-8	23 (71.87)	12.68	19 (82.6%)	4 (17.39%)
	9-15	6 (18.75)	13.86	4 (66.66%)	2 (33.33%)
	16-22	3 (9.37)	13.96	1 (33.33%)	2 (66.66%)

The demographic characteristics of the study subjects were given in Table 1. In this study the mean CBMN frequency (13.96) and incidence of Karyotype (66.66%) were increasing with increasing age. As the birth order of the study subjects increased the mean CBMN frequency also increased. In the case of karyotype analysis all range of birth order showed equal distribution of abnormal karyotype. The mean CBMN frequency (13.67) and incidence of abnormal karyotype (70%) were increased in subjects residing in urban area. Subjects who had sedentary type of occupation showed high mean CBMN frequency (13.69) as well as high incidence of abnormal karyotype (61.11%). In the case of subjects with non-sedentary type of occupation, they showed mean CBMN frequency of 12.52 and also 28.57% incidence of abnormal karyotype. While considering the duration of married life, the mean CBMN frequency and abnormal karyotype increases with duration of married life becomes longer.

Distribution Of Mean Cbmn Frequency And Karyotype According To Physiological Characteristics Of Study Subjects
Table 2:

Variables	Category	Number (%)	Mean CBMN frequency	Normal karyotype	Abnormal karyotype
Family H/o infertility or subfertility	No	29 (90.62)	12.96	25 (86.20%)	4 (13.79%)
	Yes	3 (9.37)	13.96	0	3 (100%)
Family h/o Cancer among first and second degree relatives	No	28 (87.5)	12.89	24 (85.71%)	4 (14.28%)
	Yes	4 (12.5)	14.17	1 (25%)	3 (75%)
Semen analysis	Azoospermia	5 (15.62)	14.4	1 (20%)	4 (80%)
	Oligozoospermia	13 (40.62)	13.24	10 (76.92%)	3 (23.07%)
	Normozoospermia	14 (43.75)	12.39	13 (92.85%)	1 (7.14%)

Table 2 represents the physiological characteristics of the study subjects. The study subjects with family history of infertility or subfertility showed high mean CBMN frequency of 13.96 and also showed high incidence of abnormal karyotype. Subjects those with family history of cancer among first and second degree relatives showed high mean CBMN frequency of 14.17 and also showed high incidence of abnormal karyotype (75%). Distribution of mean CBMN frequency and karyotype were analyzed according to semen analysis. 43.75% of subjects had normal sperm count with an incidence of 7.14% abnormal Karyotype. 15.62% of subjects were azoospermic and showed a mean CBMN frequency of 14.4. In the case of azoospermic subjects an incidence of 80% showed abnormal karyotype. Among 32 subjects, 40.62% subjects were oligozoospermic and showed a mean CBMN frequency of 13.24 with an incidence of 23.07% abnormal karyotype.

Distribution Of Mean Cbmn Frequency And Karyotype According To Lifestyle Characteristics Of Study Subjects
Table 3:

Variables	Category	Number (%)	Mean CBMN frequency	Normal karyotype	Abnormal karyotype
History of X-ray exposure	No	29 (90.62)	13	23 (79.31%)	6 (20.68%)
	Yes	3 (9.37)	13.75	1 (33.33%)	2 (66.66%)
Parental consanguinity	No	27 (84.37)	12.17	22 (81.48%)	5 (18.51%)
	Yes	5 (15.62)	13.54	2 (40%)	3 (60%)
Smoking	No	22 (68.75)	13	19 (86.36%)	3 (13.63%)
	Yes	10 (31.25)	13.56	6 (60%)	4 (40%)
Chewing	No	28 (87.5)	12.45	23 (82.14%)	5 (17.85%)
	Yes	4 (12.5)	13.75	1 (25%)	3 (75%)
Alcohol consumption	No	25 (78.12)	12.83	23 (92%)	2 (8%)
	Yes	7 (21.87)	13.82	2 (28.57%)	5 (71.42%)

Lifestyle characters of the study subjects were given in table 3. Among 32 study subjects, 3 subjects had history of X-ray exposure and showed high mean CBMN frequency of 13.75 and also higher incidence of abnormal karyotype (66.66 %). While considering the parental consanguinity, 5 of them with parental consanguinity had a mean CBMN frequency of 13.54 and out of 5 subjects with parental consanguinity 3 of them showed abnormal karyotype. In the present study, 10 subjects with smoking habit showed mean CBMN frequency of 13.56 and among them the incidence of abnormal karyotype were 40%. Subjects with habit of chewing showed high mean CBMN frequency of 13.75 and more incidence of abnormal karyotype (75%) than those without the habit of chewing. In the case of habit of alcohol consumption, 7 subjects with this habit (21.87%) showed high mean CBMN frequency of 13.82 and higher incidence of abnormal karyotype (71.42%).

Distribution Of Mean Cbmn Frequency And Karyotype According To Biochemical Parameters Of Study Subjects
Table 4:

Variables	Category	Number (%)	Mean CBMN frequency	Normal karyotype	Abnormal karyotype
Total Cholesterol	≤200 mg/dl	4 (12.5)	10.15	3 (75%)	1 (25%)
	>200 mg/dl	28 (87.25)	13.47	21 (75%)	7 (25%)

Fasting Blood Sugar	80-110 mg/dl	6 (18.75)	11.3	5 (83.3%)	1 (16.66%)
	>110 mg/dl	26 (81.25)	13.46	19 (73.07%)	7 (26.92%)
Urea	≤15 mg/dl	2 (6.25)	13.14	1 (50%)	1 (50%)
	>15 mg/dl	30 (93.75)	12.01	23 (76.66%)	7 (23.33%)

The distribution of mean CBMN frequency and karyotype according to biochemical parameters are given in table 4. Out of 32 study subjects, 28 had total cholesterol level >200mg/dl with a mean CBMN frequency of 13.47 and 25% incidence of abnormal karyotype. Rest of the study subjects (≤200mg/dl) had a mean CBMN frequency of 10.15 with 25% incidence of abnormal karyotype. The extent of somatic DNA damage increased with increasing levels of total cholesterol. In the case of fasting blood sugar the higher mean CBMN frequency (13.46) were found in subjects with FBS level >110 mg/dl. Among them an incidence of 26.92% showed abnormal karyotype. The least mean CBMN frequency was 11.3 with FBS level ≤110 mg/dl and the incidence of abnormal karyotype was found to be 16.66%. A higher mean CBMN frequency of 13.14 and an incidence of 50% abnormal karyotype were found in subjects with urea level >15 mg/dl. The least mean CBMN frequency was 12.01 with urea level ≤15mg/dl and the incidence of abnormal karyotype was found to be 23.33%.

DISCUSSION:

According to Isiah et al, (2011) aging has a significant impact on male sexual function, sperm parameters and fertility, which all contribute to decreased fecundability, increased time to conception and increased miscarriage rates. Ferlin et al, (2007) reported that evidence for decline in men's fertility with increasing age. Kleiman et al, (2012) observed that aging has a significant impact on male sexual function, sperm parameters and fertility. These changes contribute to decreased fecundability and increased time to conception. The present study also indicated that the mean CBMN frequency and incidence of abnormal karyotype were increasing in subjects with increasing age.

According to Hosseinzadeh et al, (2007) cigarette smoking negatively affects sperm count, motility, and normal morphology. Saleh et al, (2002) showed that oxidative stress status in semen of smoker men is significantly higher than nonsmoker men. Reactive Oxygen Species produced by cigarette smoke induces phagocyte cells or abnormal spermatozoa causes oxidative damage to normal sperm DNA, protein and lipids, which may be closely related to sperm dysfunction (Aitken and Baker, 2006). In the present study, the mean CBMN frequency and incidence of abnormal karyotype of subjects with habits of smoking (13.56) was higher than that of subjects without habit of smoking (13).

Dhawan and Sharma (2002) reported that alcohol consumption seems to alter sperm parameters and testicular pathology. As far as sperm parameters, the more frequently abnormality reported in the higher percentage of morphologically abnormal spermatozoa. In addition, decrease in the seminal fluid leukocyte concentration has been reported. In the present study 7 subjects had drinking habit and showed high mean CBMN frequency (13.82) and high incidence of abnormal karyotype than that of subjects without habit of alcohol consumption (12.83).

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 radiation in the epididymis. Thonneua et al, (1998) sperm-function tests including sperm kinematics and sperm fertilizing capacity also were affected significantly by the small increase in the radiation. In the present study mean CBMN frequency was higher (13.75) than that of subjects without X-ray exposure.

Ola Faris Al-Quzwini et al, (2016) reported that occupational hazard is associated with oligozoospermia or teratozoospermia. Jobs that require working in hot environments or mechanical trauma and physical load on the pelvic contents can reduce semen quality. In the current study subjects with sedentary occupation showed a high mean CBMN frequency of 13.69 and greater incidence of abnormal karyotype.

In the present study subjects with parental consanguinity had significant increase of mean CBMN frequency and incidence of abnormal karyotype. According to Rita et al, (2000) consanguineous marriages, a cultural practice governed by consanguinity values and norms, increase a couple's risk of enduring the death of one or more of their children. According to Zlotogora, (2006) in communities with a high level of consanguineous marriage, diagnosis of a recessive disorder in one or more members of the same family is generally indicative of a recent mutation, whereas the presence of a rare disorder in several families suggests an older mutational event or previous admixture through marriage with a person from another community.

Chin Yu Liu et al, (2017) demonstrated that the total cholesterol level was positively correlated with total sperm motility and progressive motility with statistical significance. The life style characteristics of the subjects found that high serum levels of total cholesterol, free cholesterol and phospholipids were statistically associated with a low percentage of spermatozoa with intact acrosomes and a smaller sperm head area and perimeter. In the present study, the subjects with higher total cholesterol level (TC >200mg/dl) showed high mean CBMN frequency (13.47) than that of subjects with normal total cholesterol level (TC ≤ 200mg/dl).

A correlation between sperm count and pregnancy has been reported in various studies (Guzick et al, 2001; Bonde et al, 1998) showing evidence of a predictive value of sperm count. Subfertility is a common condition affecting at least 15% of couples during their reproductive lives, and in half of these, a male factor is involved (WHO, 1983); it is always defined by the finding of an abnormal semen analysis. Nwafia et al, (2006) reported that the semen analysis of one thousand one hundred and ten (1,110) males attending infertility at the University of Nigeria Teaching Hospital (UNTH) Enugu, Eastern Nigeria showed that the aetiology of male infertility in the population seem to be unrelated to sperm but related to sperm count, motility and morphology. In the present study, 5 subjects had azoospermia condition with a high mean CBMN frequency of 14.4 and higher incidence of abnormal karyotype. The current study showed a significant relationship between semen quality and infertility.

CONCLUSION:

In the present study mean CBMN frequency and abnormal karyotype were dependent variable with age, birth order, duration of married life, sedentary occupation, and various lifestyle habits. The mean CBMN frequency and incidence of abnormal karyotype showed an association with different demographic, physiological, lifestyle and biochemical characteristics. Avoid modern lifestyles such as sedentary occupation, habit of alcohol consumption, smoking, chewing and fast food consumption and taking precaution about environmental factors such radiation exposure and temperature at workplace will help to minimize the risk for male infertility. Ideal marriage age, absence of addictions, timely medical assistance can help couples to become pregnant successfully.

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