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SOMATIC DNA DAMAGE ASSOCIATED WITH OXIDATIVE STRESS AND IMMUNE RESPONSE IN SUBFERTILITY

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Abstract

Subfertility (also commonly known as infertility), which affects one in six couples, is the failure to manifest a clinical pregnancy after 12 months of regular, unprotected intercourse. Even though genetic, environmental and lifestyle factors play a pivotal role in subfertility, the etiology of the disease still remains enigmatic. The extent of somatic DNA damage and oxidative stress among subfertile subjects was studied. TORCH infection screening and several clinical parameters were also analyzed among 74 subfertile females and 45 age and sex-matched healthy individuals as control. The study concluded that subjects with subfertility showed increased oxidative stress and associated somatic DNA damages along with varying degrees of infections.

Keywords: Subfertility, DNA damage, Mean CBMN frequency, Karyotype, Oxidative Stress

INTRODUCTION

Subfertility is usually defined on a pragmatic basis as failure to conceive after one year of regular unprotected intercourse or the occurrence of two or more consecutive natural miscarriages or stillbirths (Gnoth et al 2005). The term subfertility describes any form of reduced fertility with a prolonged time of unwanted non-conception and includes many reversible causes Gnoth et al (2005). Adamson and Baker (2003) suggested that, "common causes of subfertility include ovulatory disorders, tubal disease, peritoneal adhesions, endometriosis, uterine abnormalities, abnormalities of sperm and advancing female age". In 2016, Coutton et al estimated that, "the overall burden of subfertility/infertility is significant, likely underestimated and has not displayed any decrease over the last 20 years".

According to Nickerson et al (2012), "the prenatal and perinatal infections, falling under the designation of TORCH complex, are a medical acronym for a set of perinatal infections, i.e., infections that are passed from a pregnant woman to her fetus". Maldonado et al (2011) denoted that, "the TORCH agents—*Toxoplasma gondii*, *Rubella virus*, *Cytomegalovirus* (CMV) and *Herpes simplex virus* (HSV)—are the most common infectious agents causing asymptomatic or mild infection in the mother but much more serious consequences in the fetus". Kumari et al (2011) explained that, "infections caused by TORCH and others agents like *Chlamydia trachomatis*, *Treponema pallidum*, *Neisseria gonorrhoeae*, HIV, etc. are the major causes of Bad

Obstetric History (BOH)". Sadik et al (2012) mentioned that, "BOH implies previous unfavourable fetal outcome in terms of two or more consecutive spontaneous abortions, history of intrauterine fetal death, intrauterine growth retardation, stillbirth, early neonatal death, and/or congenital anomalies".

Farag and Alagawany (2018) suggested that, "the effect of several factors (environmental, synthetic chemicals, UV rays, genetic defects) on DNA modulates its functionality within cells and ultimately results in notable changes in the living organisms". Salar et al (2017a) denoted that, "the majority of DNA damage occurs in human beings in response to oxidative stress (OS). Several factors are responsible for the formation of reactive oxygen species (ROS) and free radicals (FRs)". Toyokuni (2006) explained that, "these by-products are accepted as sole factors for OS-mediated injuries in living organisms. The frequency of cellular DNA damage in humans depends on the type and quantity of bioactive constituents production in response to FRs". Das and Roychoudhury (2014) denoted that, "generation of ROS and OS conditions results in modification of DNA bases which leads to abnormality (mutations, translocations, gene inactivation) at genomic level".

Recent studies establish that genetic factors as well as lifestyle and environment factors play a crucial role in subfertility. Even though, the pathophysiological reason behind subfertility remains unexplained. No systematic studies were conducted so far to evaluate the extent of oxidative stress and subsequent somatic DNA damages associated with subfertile subjects having varying degrees of infections, other lifestyle and environmental risk factors. Hence, the present study was undertaken to evaluate the role of somatic DNA damage associated with oxidative stress and immune response among subfertility subjects. The specific objective of the study is to evaluate the chromosomal abnormalities, if any, present in women experiencing subfertility by karyotype analysis using peripheral blood lymphocyte culture method described by Moorhead et al (1990), and GTG banded karyotypes were prepared according to ISCN pattern 1995. The extent of the somatic DNA damages were analysed by Cytokinesis-block micronuclei (CBMN) assay proposed by Fenech in 1993.

MATERIALS AND METHODS

Seventy four female subjects suffering from subfertility were selected as test subjects. Forty five age and sex matched subjects were selected as controls. Detailed demographic, physiological and lifestyle characteristics of the subjects were recorded using proforma. Eight ml of venous blood was collected aseptically from all the subjects by venipuncture after overnight fasting. Four 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 plain tubes for other investigations. Biochemical tests like Fasting Blood Sugar (FBS), Triglyceride, Total Cholesterol, HDL Cholesterol and LDL Cholesterol were performed. Oxidative stress marker [Malondialdehyde (MDA)] and inflammatory marker [high sensitivity C Reactive Proteins (hsCRP)] were analyzed among the study subjects. Immunological evaluation was performed by TORCH Screening.

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OBSERVATIONS AND RESULTS

Subjects' ages ranged from 18 to 45 years. The mean age of test subjects was 34.90 ± 3.87 and 33.31±5.48 for control. The difference in mean age between groups found no statistical significant (t = 1.853; p= 0.03).

Table.1: Distribution of mCBMNF and karyotype among test and control subjects

Variables	Number	Mean CBMN frequency	Normal Karyotype (%)	Abnormal Karyotype
Test	74	12.8	69 (93.24%)	5 (6.76%)
Control	45	10.32	45 (100%)	0

The mean CBMN frequency of the test subjects was 12.8 and 10.32 for control subjects. There is statistically significant difference between the mCBMNF of test and control subjects was observed with a p value <0.001 (t=13.74). The observed incidence of abnormal karyotype among test subjects was 6.76% (n=5).

Table.2: Distribution of TORCH Positivity among study subjects

Infections	Seropositivity	Negative
CMV IgG	22	52
Toxoplasma IgG	27	47
Rubella IgG	22	52
HSV IgG	21	53
Other Infections	15	59

TORCH screening was performed and the majority of the study subjects were Toxoplasma IgG positive.

Table.3: Distribution of mean CBMN frequency and karyotype according to TORCH infections

Infections	Seropositivity / Negative	Number	Mean CBMN frequency	Normal Karyotype (%)	Abnormal Karyotype (%)
Toxoplasma	IgG positive	27	13.01	23 (85.1%)	4 (14.9%)
	Negative	47	12.68	46 (97.8%)	1 (2.2%)
Rubella	IgG positive	22	12.87	19 (86.3%)	3 (13.7%)
	Negative	52	12.77	50 (96.1%)	2 (3.9%)
CMV	IgG positive	22	13.28	21 (95.4%)	1 (4.6%)

	Negative	52	12.6	48 (%)	4 (%)
HSV	IgG positive	21	12.67	19 (90.4%)	2 (9.6%)
	Negative	53	12.85	50 (94.3%)	3 (5.7%)
Other Infections	IgG positive	15	13.51	14 (93.3%)	1 (6.7%)
	Negative	59	12.62	55 (93.2%)	4 (6.8%)

Subjects reported with TORCH infection showed an increased mean CBMN frequency than the rest. The observed incidence of abnormal karyotype among test subjects with positivity of Toxoplasma, Rubella, CMV, HSV and other infections were 14.9%, 13.7%, 4.6%, 9.6% and 6.7% respectively.

Table.4: Distribution of mean CBMN frequency and karyotype according to Demographic characteristics

Variables	**Category**	**Number**	**Mean CBMN frequency**	**Normal Karyotype** (%)	**Abnormal Karyotype** (%)
Age	≤30	10	12.79	9 (90%)	1 (10%)
	>30	64	12.8	60 (93.75%)	4 (6.25%)
Residence	Urban	45	12.86	42 (93.34%)	3 (6.66%)
	Rural	26	12.76	25 (96%)	1 (4%)
	Coastal	3	12.21	2 (66.67%)	1 (33.33%)
Occupational Type	Sedentary	31	12.93	30 (96%)	1 (4%)
	Non Sedentary	43	12.7	39 (86.66%)	3 (13.34%)
Socioeconomic status (SES)	High	7	12.59	6 (85.71%)	1 (14.29%)
	Average	52	12.77	49 (94%)	3 (6%)
	Low	15	12.99	14 (93.33%)	1 (6.67%)
Age at marriage	≤25	58	12.73	54 (93%)	4 (7%)
	>25	16	13.06	4 (80%)	1 (20%)
Duration of married life (years)	≤10	40	12.57	38 (95%)	2 (5%)
	>10	34	13.07	31 (91%)	3 (8%)

The age of the test subjects were grouped into two as ≤30 and >30 years. The observed mean CBMN frequency of test subjects with advanced age (>30 years) was 12.8 and their observed incidence of abnormal karyotype was 6.25% (n=4). The mean CBMN frequency and karyotype were analysed based on the place of residence. The test subject who resides in urban area showed an increased mean CBMN frequency (12.86) than the rest. The occupational type of the subjects was categorised as sedentary and non-sedentary and their observed mean CBMNF was 12.93 and 12.7 respectively. Based on the SES, age at marriage and duration of married life (years) the observed mean CBMNF of test subjects was 12.77,

12.73 and 12.57 respectively. Moreover, the observed incidence of normal and abnormal karyotype was also mentioned in the table.no:4.

Table.5: Distribution of mean CBMN frequency and karyotype according to Clinical conditions

Variables		Number	Mean CBMN frequency	Normal Karyotype (%)	Abnormal Karyotype (%)
History of (H/o) Diabetes	Yes	15	13.05	14 (93.3%)	1 (6.7%)
	No	59	1.74	55 (93.2%)	4 (6.8%)
H/o Hypertension	Yes	9	12.94	8 (88.8%)	1 (11.2%)
	No	65	12.78	61 (93.8%)	4 (6.2%)
H/o Dyslipidemia	Yes	16	13.36	14 (87.5%)	2 (12.5%)
	No	58	12.64	55 (94.8%)	3 (5.2%)
H/o Chronic illness	Yes	6	13.22	6 (100%)	0
	No	68	12.76	63 (92.6%)	5 (7.4%)
H/o Thyroid disorder	Yes	11	13.14	9 (81.8%)	2 (18.2%)
	No	63	12.74	60 (95.2%)	3 (4.8%)
H/o Infection	Yes	40	12.89	36 (90%)	4 (10%)
	No	34	12.7	33 (97%)	1 (3%)
H/o Endometriosis	Yes	14	12.86	13 (92.8%)	1 (7.2%)
	No	60	12.79	56 (93.3%)	4 (6.7%)
H/o UTI	Yes	14	12.87	13 (97%)	1(3%)
	No	60	12.78	56 (93.3%)	4 (6.7%)
Family H/o CAD	Yes	16	13.06	15 (93.75%)	1 (6.25%)
	No	58	12.73	54 (93.1%)	4 (1.07%)

The subjects reported with clinical conditions like, H/o Diabetes, H/o Hypertension, H/o Dyslipidemia, H/o chronic illness, H/o Thyroid disorder H/o Infection, H/o Endometriosis H/o UTI and H/o CAD in family showed an increased mean CBMN frequency. The observed incidence of abnormal karyotype among subjects reported with H/o Thyroid disorder was 18.2% (n=11). In addition to that, the observed incidence of abnormal karyotype among test subjects with H/o Dyslipidemia, H/o Hypertension and H/o Infection was 12.5% (n=16), 11.2% (n=9) and 10% (n=40) respectively.

Table.6: Distribution of mean CBMN frequency and karyotype according to Physiological characteristics

Physiological characters	Category	Number	Mean CBMN frequency	Normal Karyotype (%)	Abnormal Karyotype (%)
Obesity	Yes	38	12.93	33 (91.6%)	3 (8.4%)
	No	36	12.66	36 (94.7%)	2 (5.3%)
Age at menarche	≤14	66	12.83	61 (92%)	5 (8%)
	>14	8	12.53	8(100%)	0
Menstrual periods	Regular	36	12.51	34 (94%)	2 (6%)
	Irregular	38	13.08	35 (92%)	3 (8%)
Number of previous abortions	≤3	33	12.41	32 (96.9%)	1 (3.1%)
	>3	41	13.11	37 (90.2%)	4 (9.8%)

Subjects with obesity showed a mean CBMN frequency of 12.93 and an incidence of observed abnormal karyotype was 8.4% (n=3). The observed incidence of abnormal karyotype among test subjects with irregular menstrual period was 8% (n=3) and their observed mCBMNF was 13.08. In the case of number of previous abortions, subjects with more than 3 abortions showed an increased mean CBMN frequency (13.11). Moreover, the observed incidence of abnormal karyotype (9.8%) was more among subjects with >3 times of abortion.

Table.7: Distribution of mean CBMN frequency and karyotype according to lifestyle factors

Lifestyle factors	Category	Number	Mean CBMN frequency	Normal Karyotype (%)	Abnormal Karyotype (%)
Dietary Pattern	Non Vegetarian	61	12.94	56 (91.8%)	5 (8.2%)
	Vegetarian	13	12.14	13 (100%)	0
Parental Consanguinity	Yes	7	13.22	6 (85.7%)	1 (14.3%)
	No	67	12.76	63 (94%)	4 (6%)
Physical activity	Good	7	12.43	6 (85.7%)	1 (14.3%)
	Average	30	12.81	27 (90%)	3 (10%)
	Poor	37	12.86	36 (97.2%)	1 (2.8%)
Water intake per day	Good	14	12.5	14 (100%)	0
	Average	35	12.7	30 (85.7%)	5 (14.3%)
	Poor	25	13.1	25 (%)	0
Alcohol consumption	Yes	2	12.82	2 (100%)	0
	No	67	12.16	62 (92.5%)	5 (7.5%)

An elevated mCBMNF was observed among test subjects following non-vegetarian diet when compared to the rest. Subjects reported with parental consanguinity showed an increased mCBMNF (13.22) and their observed incidence of abnormal karyotype (14.3%). Those who reported with poor water intake per day, poor physical activity and with a habit of alcohol consumption showed an increased mCBMNF when compared to the rest. The observed incidence of karyotype was also mentioned in the table.no:7.

Table.8: Distribution of mean CBMN frequency and karyotype according to Biochemical Characteristics

Biochemical characters	Value	Number	Mean CBMN frequency	Normal Karyotype (%)	Abnormal Karyotype (%)
FBS (mg/dL)	≤110	58	12.76	54 (93.1%)	4 (6.9%)
	>110	16	12.93	15 (937%)	1 (6.3%)
Total Cholesterol (mg/dL)	≤200	51	12.59	48 (94.1%)	3 (5.9%)
	>200	23	13.27	21 (91.3%)	2 (8.7%)
HDL-C (mg/dL)	≤40	36	12.98	33 (91.6%)	3 (8.4%)
	>40	38	12.63	36 (94.7%)	2 (5.3%)
LDL-C (mg/dL)	≤100	6	12.73	5 (83.3%)	1 (16.7%)
	>100	68	12.81	64 (94.1%)	4 (5.3%)
Triglyceride (mg/dL)	≤150	37	12.75	35 (94.5%)	2 (5.5%)
	>150	37	12.85	34 (91.8%)	3 (8.2%)

Subjects with FBS concentration >110 mg/dL showed an increased mCBMNF of 12.93 than the rest. The observed incidence of abnormal karyotype among test subjects with FBS level >110 mg/dL was 6.3% (n=1) and for subjects with FBS level ≤110 mg/dL the observed abnormal karyotype was 6.9% (n=4). Test subjects with total cholesterol level >200 mg/dL showed an elevated mCBMNF of 13.27 and their observed incidence of normal karyotype and abnormal karyotype were 91.3% (n=21) and 8.7% (n=2) respectively.

Subjects with HDL-C level of ≤40 mg/dL showed an increased mCBMNF of 12.98. Subjects with increased LDL-C level (>100 mg/dL) showed an elevated mCBMNF of 12.81. Half of the test subjects with triglyceride level >150 mg/dL showed increased mean CBMN frequency 12.85 and their observed incidence of abnormal karyotype observed was (n=3) 8.2%.

Table.9: Distribution of mean CBMN frequency and karyotype according to level of oxidative stress marker and inflammatory marker

Variables	Category	Number	Mean CBMN frequency	Normal Karyotype (%)	Abnormal Karyotype (%)
MDA (µmol/L)	≤2.5	66	12.74	61 (92.4%)	5 (7.6%)
	>2.5	8	13.26	8 (100%)	0
hsCRP (mg/L)	≤2	48	12.79	44 (91.6%)	4 (8.4%)
	>2	26	12.81	25 (96.1%)	1 (3.9%)

Test subjects with MDA concentration >2.5 µmol/L showed a mCBMNF of 13.26 and the rest with MDA level ≤2.5 µmol/L showed a mCBMNF of 12.74. The observed incidence of abnormal karyotype was mentioned in the table.no.9. Test subjects with hsCRP level >2mg/dL showed an elevated mCBMNF (12.81) compared to the rest. The observed incidence of normal and abnormal karyotype was also mentioned in the table.no: 9.

DISCUSSION

In the current study observed an increase no. of abortions among subjects with advanced maternal age. This was supported with Rocherbrochard and Thonneau (2002) who reported that, "advanced maternal age has been associated with increased number of miscarriages and more than 40% study subjects were above the age of 35 years". According to De et al (2015), "maternal age and previous miscarriage rates increases the risk of subsequent miscarriages". In 2004, Harper denoted that, "fertility declines with advancing maternal age which increases miscarriage rates and chromosomal abnormalities".

In the present study, incidence of abnormal karyotype among study subjects with age >30 years was 6.25% (n=4). According to Minsa et al (2016) it was mentioned that, "increased micronuclei frequency for abnormal level of biochemical and hormonal investigations. The cytokinesis-block micronuclei assay revealed an increased micronucleus frequency in couples with infertility or two or more spontaneous abortions, suggesting a possible role of chromosomal instability in reproductive failure". The current study observed an elevated mCBMNF was observed among test subjects.

The current study analysed the role of infection and seroprevalence among subjects with subfertility. Moreover, subjects reported with TORCH infection showed an increased mean CBMN frequency than the rest. Shrivastava et al (2014) explained that, "in India, the reported seroprevalence rate of toxoplasmosis is up to 80%". Gandhoke et al (2007) estimated that, "globally, the reported prevalence of CMV infection is ranging from 45% in developed countries to 100% in developing countries, whereas in India, recorded 80%–90% CMV IgG antibodies in women of child-bearing age". Mohammad and Salman (2014) observed that, "Toxoplasma antibodies which is a known etiological agent in recurrent pregnancy wastage was found in 26.83% of women with BOH". In the present study, the role of infections among subjects with subfertility and the seroprevalence among subjects with subfertility was analysed.

In current study, subjects reported with obesity and chronic illness showed high mean CBMN frequency of 12.93 and 13.22 respectively. Lampic et al (2006) reported that, "the increased rates of obesity and medical illness found in reproductive-aged women, intentionally delayed childbearing". In 2012, Jungheim suggested that, "the relationship between obesity and reproductive functions has been known for many years and it is still being explored". In the present study it was observed that, majority [n=38 (52%)] of the study subjects were reported with obesity. In addition to, these subjects showed various biochemical and physiological risk factors.

Current study observed that, an increased mCBMNF of 13.08 and 13.11 in subjects with delayed menarche and more no. of previous abortions. Ajeet (2014) reported that, "menstrual irregularities in the form of any deviation from normality like, oligomenorrhea, hypo or hypermenorrhagia were also significant risk factors for primary infertility". In the present, subjects with irregular menstrual cycle (51.3%) showed an elevated mCBMNF of 13.08 than the subjects with regular menstrual cycle. Arun et al (2015) revealed that, "females with the age of menarche more than 15 years were more risky to develop infertility than those with age of menarche less than 15 years".

Current study observed mCBMNF among subjects with H/o thyroid disorders. This was similar to study done by Meenakshi Titoria Sahu et al (2010) who reported that, "thyroid disorders are one among the common endocrine problems in pregnant women. However, subclinical thyroid dysfunction also has adverse effects on maternal and fetal outcomes".

In the present study it was observed that, in total of 74 test subjects, seven showed parental consanguinity with high mean CBMN frequency and chances of abnormal karyotype. According to Zlotogora, (2010), "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".

In the current study, test subjects with increased level of FBS concentration (>110 mg/dL), high TC, high TG, high LDL-C and hsCRP level showed an elevated mCBMNF. Whereas, test subjects with low concentration of HDL-C showed an increased mCBMNF. This was in accordance with the study done by Minsa, (2016), who reported that, increased mean CBMN frequency was found among subjects with increased level of biochemical factors like FBS, TC, TG and LDL-C". Verit et al in 2017 found that, "TG, TC, LDL and hsCRP levels were elevated and HDL was decreased in women with unexplained infertility".

In the current study also an increased level of MDA concentration was observed among the test subjects when compared to the control. This was supported by Dong et al (2001) who suggested that, "OxS biomarkers have been found in various sites in the female reproductive tract, suggesting their role in various physiological functions". In the study by Veena et al (2008) reported, "an elevated MDA levels in serum of infertile women, than in fertile women signifies that the oxidative damage in infertile women".

CONCLUSIONS

The present study concluded that subjects with subfertility showed an increased oxidative stress and subsequent somatic DNA damages. In addition to, subjects with varying degrees of infection and other lifestyle associated risk factors also showed an increased somatic DNA damages. It is advised to consider a previous history of pregnancy loss and the serological reactions for torch infection while managing BOH to reduce the adverse fetal outcome. It is also important to understand the ways in which lifestyle behaviours and biochemical parameters may benefit or harm the reproductive health and pregnancy. By actively modifying these variables, men and women might be capable of controlling their own fertility potential. Timely medications and early diagnosis of chromosomal anomalies will reduce pregnancy wastages.

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