Cardiovascular Risk Markers in Infertile Subjects

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Abstract

A study was conducted to evaluate the cardiovascular risk markers in infertile subjects. There were 64 study subjects in the age group of 19 to 50 years and 40 age matched healthy controls. There are some data suggesting that infertility have an impact on the risk of Cardiovascular disease; the present study was carried out to evaluate the various risk factors, if any, among infertile subjects for cardiovascular disease and to quantify the extent of somatic DNA damage by Cytokinesis-Block Micronuclei assay and the results were correlated with various demographic (age, birth order, residence, religion, education, etc), physiological (diabetes, hypertension, dyslipidemia, semen analysis, abdominal obesity, polycystic ovarian syndrome, etc) and lifestyle characteristics (history of chronic illness, smoking, drinking, consanguinity, duration of married life, etc). A significantly elevated level of micronuclei (MN) in the study subjects along with the above parameters is suggestive of increased risk of cardiovascular disease in infertile subjects. Modification of the lifestyle like dietary habits, exercise and changing sedentary life style is recommended for reducing the risk for cardiovascular diseases.

Key Words: Cardiovascular diseases, CBMN (Cytokinesis-Block Micronucleus) assay, Dyslipidemia, Diabetes, DNA damage, Infertility, Polycystic Ovarian Syndrome.

Introduction

Cardiovascular disease refers to any disease that affects the cardiovascular system, principally cardiac disease, vascular diseases of the brain and kidney, and peripheral arterial disease [1]. Cardiovascular disease is the leading cause of deaths worldwide, [2, 3] and have increased at a fast rate in low and middle-income countries [4]. Subfertility shares common pathways with cardiovascular disease (CVD), including polycystic ovarian syndrome [5], obesity and thyroid disorders. Women with infertility are at an increased risk of incident CVD when compared with fertile women [6]. Infertility is defined by the American society for reproductive medicine (ASRM) as a disease of the reproductive system that impairs the body's ability to perform the basic function of reproduction [7]. According to WHO, 8 – 12 % of couples around the world experiences difficulty in conceiving a child [8, 9], i.e., 60-80 million couples worldwide currently suffer from infertility [10].

Cardiovascular disease (CVD) is the number-one killer of women. Women with primary ovarian insufficiency (POI) may be more burdened by cardiovascular disease, such as myocardial infarction and stroke, as compared with women with normal menopause. The increased burden may be mediated by a worsening of cardiovascular risk factors, such as lipids, corresponding with the loss of ovarian function. Almost all CVDs, in women occur after the menopause. Nonetheless, anovulation and infertility have an impact on the risk of CVD. Couples with primary infertility have never been able to conceive, while, on the other hand, secondary infertility is difficulty in conceiving after already having conceived (and either carried the pregnancy to term or had a miscarriage). There are some data suggesting that irregular menstrual cycles are associated with an increased risk of myocardial infarction in young women; [11] however, residual confounding by socioeconomic status or other correlates of the CVD risk cannot be excluded. The association between infertility, anovulation and CVD risk is moderate and hence of limited, if any, relevance for an individual risk assessment. It is even more complex to understand a possible role of infertility on the risk of CVD. Consequently, the few earlier studies show a consistent association of infertility and the risk of CVD. A prospective investigation demonstrated that menstrual irregularity, a known correlate of subfertility [12], is related to increased risks for women of cardiovascular disease (CVD) in later life [13].

Women who experience infertility have high levels of psychological stress manifesting as both depression and anxiety [14] and psychosocial stressors in turn are linked to CVD [15]. This is potentially through mechanisms related to altered immunomodulation [16] or vascular dysfunction. Thus, psychosocial stress may contribute increased risk of incident CVD among women with prolonged periods of infertility and subfertility.

Researchers found women who were unable to become pregnant for at least five years, had a 19% increased risk of heart disease, compared with women who had no problems getting pregnant. But whether such infertility or subfertility is a risk factor for cardiovascular disease still remains unclear. Most of the risk markers are induced by the changes occurred during infertility. Men with lower sperm quality have been shown to have a less healthful diet, which would also be expected to lead to more cardiovascular disease. Just as erectile dysfunction can indicate poor lifestyle habits and can be an important indicator of cardiovascular risk; it appears that male infertility also predicts future cardiovascular disease. However, the lack of adequate data makes it uncertain whether infertility leads to incident CVD independent of classic CVD risk factors. Hence the present study was undertaken to evaluate the various risk factors, if any, among infertile subjects for cardiovascular diseases and to quantify the extent of somatic DNA damage by Cytokinesis-Block Micronuclei assay in infertile subjects and to correlate the extent of somatic DNA damages with various demographic, physiological and lifestyle parameters among subjects with infertility and to assess the risk for Cardiovascular diseases.

Materials and Methods

Sixty four infertile subjects with varying cardiovascular risk markers were selected as study subjects. Forty subjects including both males and females were selected as control subjects. These subjects were referred from various infertility clinics and maternity centres of Kerala. Informed consents were obtained from all the subjects of the study according to the norms laid down by the Institutional Ethical Committee. Demographic, physiologic and lifestyle characteristics were recorded using proforma.

2-3 ml of venous blood was collected in sodium heparinised vacuutainers under strict sterile conditions from all subjects to quantify the extent of DNA damages by CBMN assay. The frequency of micronuclei among 1000 binucleated cells were counted and analysed. The

collected data were subjected to statistical analysis using statistical package for social survey (SPSS).

5 to 6 drops of whole blood samples was transferred to a vial containing 10 ml of RPMI 1640 medium supplemented with 15% foetal bovine serum. Added 10µgm/ml of phytohaemagglutinin (PHA) and incubated at 37^0C for 72 hours. After 44 hours of PHA stimulation, added cytochalasin B to the cultures to give a concentration of 4.5µgm/ml. After 28 hour addition of cytochalasin B, transferred the whole contents into a sterile centrifuge tube and centrifuged for 10 minutes at1000 rpm, removed the supernatant, shaken the pellet in a cyclomixer. Added 10 mL of 0.075M KCl solution to the cell button and kept at 37^0C for 10 minutes. After this added 2 drops of freshly prepared fixative (methanol: acetic acid in the ratio 3:1). Again centrifuged at 1000 rpm for 10 minutes and removed the supernatant, mixed the cell button in a cyclomixer and added 10 ml of freshly prepared fixative and centrifuged at 1000 rpm for 10 minutes. Repeated this process until the supernatant becomes clear and cell button becomes white. From the cell button prepared the cell suspension and 7-8 drops of cell suspension was dropped on pre cleaned, labelled and chilled slides from a particular height. The slides were flamed gently on spirit lamp, blown gently on the material and air dried. Stained the slides with 10-20% Giemsa stain solution and allowed to remain for 10 minutes. After 10 minutes the excess stain was washed off with running water and slides were air dried. The slides were examined at 100 X magnification. The number of micronuclei in number less than 1000 binucleated cells was scored and the distribution of micronuclei among binucleated cells was recorded.

Results

The demographic characteristics findings are given in the table 1 & 4. The age of the infertile husband ranged from 24 to 50 years with a mean average age of 33.84 years and the age of infertile wives ranged from 19 to 50 years with a mean average age of 27.6 years. The highest mean CBMN frequency observed among infertile husbands belonged to 35-45 years age groups and in infertile wives it was 45-55 year age group. The birth order of infertile husbands and wives ranged from 1 to 10 and the highest CBMN frequency was shown in birth order ranged from 7-9. Majority of the study subjects (65.62%) belonged to rural area followed by urban

(28.12%) and costal area (6.25%). The highest number of infertile husband and wives belongs to the religion Hindu (n=22, 68.75%) followed by Muslim (n=6, 18.75%) and Christian (n=4, 12.5%). The highest mean CBMN frequency was recorded in Muslim infertile husbands (15.38) and infertile wives (15.15). Majority of the infertile husbands were up to higher secondary (n=16, 50%) with highest mean CBMN frequency (15.36) and infertile wives were graduates (n=17, 53.12%) with highest mean CBMN frequency (15.8). The highest mean CBMN frequency was shown by the subjects belonged to Sedentary 15.59.

84.37% of infertile husbands belong to sedentary occupation with the highest mean CBMN frequency (14.83). Majority of the infertile wives comes under sedentary occupation (78.12%) with highest CBMN frequency (16.82). Majority of the infertile women had medium economic status (68.75%) and higher mean CBMN frequency was found in low economic status subjects (15.8).

The physiological characteristics are given in the table 2 & 5. The infertile husbands with diabetes (34.37%) were less in number than those without diabetes (65.62%). The highest CBMN frequency was shown by the subjects with diabetes (16.33). Among the infertile wives also the highest CBMN frequency (15.2) was shown by the subjects with diabetes (15.62%). Hypertension was reported in 43.75% of infertile husbands with high mean CBMN frequency of 15.92 and 12.5% of infertile wives reported hypertension with high CBMN frequency of 16.07. 21.87% of infertile husbands and wives were dyslipidemic subjects with high mean CBMN frequency of 16.02 and 16.6 respectively. 31.25% of infertile husbands has abdominal obesity with a high mean CBMN frequency of 15.95 and 28.12% of infertile wives has an abdominal obesity with high mean CBMN frequency of 14.55. The semen analysis of infertile husbands showed a high mean CBMN frequency (18.1) in Oligozoospermic condition. Irregular menstruation was reported in 9 out of 32 infertile wives (71.87%), with high mean CBMN frequency of 14.83. Majority of infertile women (n=29, 90.62%) had attained menarche the age range from 13-15, with high mean CBMN frequency of 14.8. It was reported that 15.6% of infertile wives has PCOS with a high mean CBMN frequency of 15.2. Family history of infertility/sub fertility was reported in 1 out of 32 infertile wives with mean CBMN frequency of 16.1 and family history of cancer in 1 out of 32 infertile wives with the high mean CBMN frequency 14.69.

The lifestyle characteristics are given in the table 3 & 6. Only one among the infertile husbands and wives had the family history of chronic illness with mean CBMN frequency of 16.3 and 16.7 respectively. Only 2 subjects among the infertile wives had the family history of X-ray exposure with high mean CBMN frequency of 17.05. History of smoking was reported in 25% of infertile husbands with high mean CBMN frequency of 16.26 and 6.25% of infertile husbands had drinking habit with high mean CBMN frequency of 16.6. Parental consanguinity was reported in 18.75% and 6.25% of infertile husbands and wives with a mean CBMN frequency of 15.23 and 15.1 respectively. The highest mean CBMN frequency was shown by the subjects who had parental consanguinity. Consanguinity was reported only in 6.25% of infertile wives with mean CBMN frequency of 14.65. Consumption of contraceptive drugs was reported in 12.5% of infertile wives with mean CBMN frequency of 15.1. The duration of married life of the infertile subjects ranged from 1 to 12 years and majority of the couples come under the range of 1 to 4 years.

The infertile husbands showed a mean CBMN frequency of 15.24 and the control husbands showed a mean CBMN frequency of 10.7. The infertile wives showed a mean CBMN frequency of 14.68 and the control husbands showed a mean CBMN frequency of 9.94. These differences showed a significant statistical difference ($p=<0.01$). Moreover a positive correlation between the number of risk markers and the extent of DNA damages was observed.

Table 1. Distribution of demographic characteristics of the infertile husbands

Distribution of demographic characteristics of the infertile husbands				
	Variable	Number	Percentage (%)	CBMN Frequency
Age	≤ 30	12	37.5	14.75
	31-40	15	46.8	15.24
	41- 50	5	15.6	16.38
Birth order	≤ 3	18	56.25	14.5
	4-6	8	25	15.4
	7-9	6	18.75	17.01
Residence	Rural	21	65.62	15.36
	Urban	9	28.12	16.15
	Costal	2	6.25	14.75
Religion	Hindu	22	68.75	15.32
	Muslim	6	18.75	15.36
	Christian	4	12.5	14.57
Education	Secondary	2	6.25	10
	Higher secondary	16	50	15.36
	Graduates/PGs	13	40.62	14.91
	Professional degree / PGs	1	3.12	16
Occupation	Sedentary	27	84.37	14.83
	Non sedentary	5	15.62	17.07

Table 2. Distribution of Physiological characteristics of the infertile husbands

Distribution of Physiological characteristics of the infertile husbands				
	Variable	Number	Percentage (%)	CBMN Frequency
Diabetes	No	21	65.62	14.6
	yes	11	34.37	16.33
Hypertension	No	18	56.25	14.7
	yes	14	43.75	15.92
Dyslipidemia	No	25	78.12	15.02
	yes	7	21.87	16.02
Semen analysis	Normal	26	81.25	15.2
	Not known	4	12.5	14.62
	Azoospermia	1	3.12	16
	Oligozoospermia	1	3.12	18.1
Abdominal obesity	No	22	68.75	14.91
	yes	10	31.25	15.95

Table 3. Distribution of Lifestyle characteristics of the infertile husbands

Distribution of Lifestyle characteristics of the infertile husbands				
	Variable	Number	Percentage (%)	CBMN Frequency
H/o chronic illness	No	31	96.87	15.2
	yes	1	3.12	16.3
Smoking	No	24	75	14.9
	yes	8	25	16.26
Drinking	No	30	93.75	15.5
	yes	2	6.25	16.6
Parental consanguinity	No	26	81.25	15.14
	yes	6	18.75	15.23

Table 4. Distribution of demographic characteristics of the infertile wives

Distribution of demographic characteristics of the infertile wives				
Age	Variable	Number	Percentage (%)	CBMN Frequency
	≤ 30	25	78.12	14.70
	31-40	7	21.8	14.62
Birth order	≤ 3	19	59.3	14.9
	4-6	10	31.25	14.04
	7-9	3	9.37	15.5
Residence	Rural	21	65.62	14.39
	Urban	9	28.12	15.24
	Costal	2	6.25	15.5
Religion	Hindu	22	68.75	14.06
	Muslim	4	12.5	15.15
	Christian	6	18.75	14.68
Education	Secondary	1	3.12	15.8
	Higher secondary	14	43.75	14.94
	Graduates/PGs	17	53.12	14.41
Occupation	Sedentary	25	78.12	16.82
	Non-sedentary	7	31.25	14.38
Economic status	High	9	28.12	14.76
	Medium	22	68.75	14.6
	Low	1	3.12	15.8

Table 5. Distribution of Physiological characteristics of the infertile wives

Distribution of Physiological characteristics of the infertile wives				
	Variable	Number	Percentage (%)	CBMN Frequency
Diabetes	No	27	84.37	14.59
	yes	5	15.62	15.2
Hypertension	No	28	87.5	14.4
	yes	4	12.5	16.07
Dyslipidemia	No	25	78.12	14.15
	yes	7	21.87	16.6
Abdominal obesity	No	23	71.87	14.73
	yes	9	28.12	14.55
Menstrual Periods	Regular	23	71.87	14.63
	Irregular	9	28.12	14.83
Menarche	≤ 12	1	3.12	14.1
	13-15	29	90.62	14.8
	16-18	2	6.25	13.35
PCOS	Yes	5	15.62	15.2
	No	27	84.37	14.62

Table 6. Distribution of Lifestyle characteristics of the infertile wives

Distribution of Lifestyle characteristics of the infertile wives				
	Variable	Number	Percentage (%)	CBMN Frequency
Family h/o infertility or sub infertility	yes	1	3.12	16.1
	no	31	96.87	14.64
H/o cancer among 1st or 2nd degree relatives	yes	1	3.12	14.69
	no	31	96.87	14.4
H/o chronic illness	No	31	96.87	14.62
	yes	1	3.12	16.7
H/o X-ray exposure	No	30	93.75	14.53
	yes	2	6.25	17.05
Contraceptive pill used	No	28	87.5	14.62
	yes	4	12.5	15.1
Consanguinity	No	30	93.75	14.63
	yes	2	6.25	14.65
Parental consanguinity	No	30	93.75	14.66
	yes	2	6.25	15.1
Duration of married life	≤4	21	65.62	14.00
	5-8	9	28.12	15.95
	9-12	2	6.25	16.15

Discussion

An estimated 70 million couples worldwide who are infertile and more than half of these persons seek medical treatment for infertility [17]. Some estimates suggest that worldwide "between three and seven per cent of all couples or women have an unresolved problem of infertility. Many more couples, however, experience involuntary childlessness for at least one year: estimates range from 12% to 28% [18]. Early miscarriages, a potential unrecognized cause

of involuntary childlessness, can be due to hypercoaguable states / thrombophilia [19, 20]. Hypercoaguable states are associated with excess CVD risk [21], which is a risk factor for incident maternal CVD [22]. Another possible mechanistic link between subfertility and CVD is hypothyroidism, which is linked to infertility [23] and incident CVD [24]. 64 Subjects including both males and females with varying degree of associated cardiovascular risk markers were selected for this study and CBMN assay was performed. In this study, the demographic, physiological and lifestyle factors was concerned primarily. The study demonstrated that the incidence of cardiovascular risk markers in infertile subjects shows a positive correlation with advancing age, duration of marital life and the incidence was varied according to birth order, religion, residential area, education, occupation, and economic status.

With recent dramatic advances in infertility treatment, age related infertility remains as one of our most difficult challenges. This issue of age with our current societal trend of increased numbers of women who delay childbearing for educational and career goals leads to increase in age related infertility [25]. In this present study, a positive correlation between the increases of CBMN frequency with advancing age was observed in both husbands and wives. The highest mean of CBMN frequency was reported by both husbands and wives who had the age of >45.

Education is more strongly associated with CVD risk factors than material status in the elderly. The best predictors of risk factors were age, sex and education. As we gain knowledge about CVD risk factors, we may be able to target preventive services in the elderly population more accurately and effectively, and help older adults make health decisions to reduce risk factors and increase their quality of life. In this study, the subjects who had completed professional degrees, and who had reported for high economic status, shows high CBMN frequency.

Lifestyle factors have had a dramatic impact on general health and the capacity to reproduce. Lifestyle issues such as smoking and obesity can affect general health and well-being. For example, smoking increases an individual's risk of cardiovascular disease and adverse consequences associated with obesity include increased risk of cardiovascular disease, diabetes and some cancers. In this study by analyzing the physiological and lifestyle characteristics it was clearly shown that the highest CBMN frequency was reported by the infertile subjects with diabetes, abdominal obesity, family history of cancer and infertility, history of chronic illness

and smoking habit. The high rate of cardiovascular risk markers in urban India compared to rural, despite low rates of smoking suggest important role of nutritional and environmental factors [26]. The present study shows high mean of CBMN frequency in urban infertile subjects who had associated cardiovascular risk markers. The Urban, rural, and coastal differences in DNA damage provide important information regarding risk factors that need prevention.

Cardiovascular disease rates in women after menopause are two to three times those of women the same age before menopause. The increased incidence of cardiovascular disease in women after the fifth decade of life coincides with the onset of menopause, which is associated with significant reduction in sex hormone, estrogen, progesterone, and androgen. However, the increase in cardiovascular risk in the fifth decade may be due to aging effects on the arteries. Infact, both men and women have increased cardiovascular risk as they age. There is no change in the slope (age vs. cardiovascular disease) as women go through menopause [26]. In the present study, the mean of CBMN frequency was higher in subjects, who had reported for delayed menarche and irregular menstrual periods.

Recent published research suggests that 50% to 75% of all Cancers and Heart Disease are caused by medical X-Rays. The research claims that more than half of all Cancers and Heart Disease are caused by medical X-Rays [10]. In this study, the male infertile alcoholics, and who had previous X ray exposure shows high range of DNA damage than others and the semen analysis of infertile husbands showed a high mean CBMN frequency in Oligozoospermic condition.

Furthermore, testosterone levels inversely correlate to degree of coronary atherosclerosis [27]. Based on a meta-analysis including 19 studies, Ruige et al found weak association between endogenous testosterone and risk for CVD in elderly but not middle-aged men and raise the question whether low androgens are a cause of CVD or, rather, a marker of a poor general health [28].

Based on the available evidence, it appears that women with POI have a higher risk of CVD and CVD mortality as compared with the general population; although this increased risk appears small. A decade ago, multiple reports concluded that young women with heart disease events had especially high mortality [29-32]. On a positive note, heart disease mortality in young women has significantly improved since the 1990s, possibly because of better recognition and

management of heart disease and its risk factors [33]. According to Manikumar (2013) more frequent CVD in PCOS are mostly mediated through increased lipids, obesity/abdominal adiposity and perhaps interacting with PCOS-related hyperandrogenism. FSH, LH, and prolactin showed a statistically significant increase in PCOS women. This type of PCOS related hyperandrogenism also contributes various clinical abnormalities leading to CVD [5]. In this study it clearly indicates that infertile wives with PCOS have high mean CBMN frequency with increased DNA damage. Thus this study clearly indicates that there is an increase in DNA damage in infertile subjects which leads to cardiovascular diseases. The lifestyle modifications will reduce the risk of cardiovascular diseases.

Conclusion

Both male and female subjects who had reported for the Cardio vascular risk markers such as, diabetes, hypertension, dyslipidemia, and abdominal obesity showed highest CBMN frequency. The level of CBMN frequency was higher among subjects who have the family history of infertility, family history of Cancer and history of chronic illness. Overall population seem to be more predisposed to Cardio vascular diseases associated with infertility. This is mainly due to the life style changes with increased consumption of high energy dense foods and decreased physical activity, and sometimes occurs as hereditary problems. Increasing awareness of the role of Genetics in the aetiology of diseases and its overall impact on the burden imposed on individuals, families and society has led to the emergence on modern clinical Cytogenetics, through this individuals can be better informed about extent of somatic DNA damages, Genetic risks and reproductive options. Another objective is to preventing the sufferings of infertile subjects with cardio vascular risk markers.

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