ISSN 0975-6299 Vol 3/Issue 2/April – June 2012

International Journal of Pharma and Bio Sciences

RESEARCH ARTICLE CYTOLOGY

FREQUENCY OF CHROMOSOMAL ABERRATIONS IN PERIPHERAL BLOOD LYMPHOCYTES OF DILATED CARDIOMYOPATHY PATIENTS

SNEHA SITARAMAN[1], THIRUMAL BABU[2] AND RADHA SARASWATHY[*1]

[1]Biomedical Genetics Research Laboratory, Division of Biomolecules and Genetics, School of Biosciences and Technology, VIT University, Vellore, India
[2]Heart line Medical and Research Centre, 72, Thennamaram Street, Vellore, India

RADHA SARASWATHY
Biomedical Genetics Research Laboratory, Division of Biomolecules and Genetics, School of Biosciences and Technology, VIT University, Vellore, India

ABSTRACT

The frequency of chromosomal aberrations (CA) in peripheral blood lymphocytes of dilated cardiomyopathy (DCM) patients was used to evaluate the extent of DNA damage and genomic instability. Blood samples were collected from 48 DCM patients of an equal number of age and sex matched controls from the Vellore region of South India. A significantly higher number of chromosomal aberrations were observed in the patient group as compared to the controls ($p<0.001$). To the best of our knowledge, this is the first report where DNA damage was estimated using the frequencies of CAs in the peripheral blood lymphocytes of dilated cardiomyopathy patients. The observation showed that it was desirable to apply CA analysis, which could be used as a biomarker for validation.

KEYWORDS

Dilated cardiomyopathy, Chromosomal aberrations, Cytogenetic analysis, Genomic instability

INTRODUCTION

Dilated cardiomyopathy (DCM) is a heterogeneous disease, clinically characterized by ventricular and sometimes atrial dilatation, with normal or reduced wall thickness, eventually leading to varying degrees of impaired systolic function. DCM represents a major health burden. There has been considerable advancement made in the understanding of the molecular genetics of cardiovascular diseases. DCM is reported to exhibit an autosomal-dominant, autosomal-recessive, X-linked, and mitochondrial patterns of transmission[1]. A number of genes have been identified in human, hamster, and mouse as causal for DCM. The gene penetrance is variable, and the clinical progression of the disease seems to be age-dependent, with a much higher incidence at older ages.

Many studies have reported DNA damage in cardiovascular disease patients by using biomarkers of DNA adducts and cytogenetic damage [2-3] No significant data exist on validating DNA damage as one of the causative agents for DCM. However, there are no reports on the cytogenetic study of DCM patients.

There is no doubt that chromosome aberration (CA) analysis is nowadays one of the best methods to ascertain the genomic damage in human genetic disorders. A number of genetic disorders with an increased spontaneous or induced chromosomal instability has been reported. It is also known that CAs are a consequence of disruption of the normal replicative and repair machinery. Damage to the DNA may lead to the formation of double strand breaks (DSBs), which if left unrepaired or misrepaired, lead to the broken chromosomes and eventually give rise to CAs[4]. Therefore, the genetic damage in the patients with DCM can be elucidated with the help of cytogenetic analysis such as, chromosomal aberration (CAs) assay using the peripheral blood lymphocytes (PBL).

Hence, in this study, an attempt has been made to validate the use of the conventional cytogenetic technique to analyse the frequency of chromosomal aberrations in DCM patients. To the best of our knowledge, this is the first cytogenetics study in PBL of patients with DCM.

MATERIALS AND METHODS

(i) Study population:

The study was carried out on 48 patients suffering from DCM and an equal number of age and sex matched controls (n=48) belonging to the Vellore region of South India. Informed written consent was obtained from all the individuals. Peripheral blood samples were collected by venipuncture in heparinized vacutainers to assess for CA frequencies. Information was collected on demographic, lifestyle and socio-economic factors. The ethical clearance was obtained from the University for conducting this study.

(ii) Cytogenetic Study:

a. Lymphocyte culture technique:

Peripheral blood samples were immediately processed and cultured according to the modified method of Hungerford[4]. Lymphocyte cultures were set up by adding 0.5 ml of whole blood with 6 ml of Ham's F10 media (PAN Biotech GmBH) supplemented with 1.2 ml of fetal bovine serum (HiMedia), 0.3 ml of phytohemagglutinin (GIBCO). The cultures were harvested after 72 hours by the addition of 2 drops of 0.001% colchicine (Sigma). The cells

were treated hypotonically with 0.075 M KCl (Merck) to lyse the red blood cells followed by fixation of the lymphocytes with methanol-acetic acid (3:1).

b. Chromosomal aberration analysis:

Fixed cells were dropped onto clean microscopic slides; air dried and stained with 4% Giemsa solution. For each individual, 50 well spread metaphases were analyzed microscopically (final magnification 100 X). The photomicrographs were taken using OLYMPUS BX51 microscope.

(iii) Statistical analysis

The significance of the difference between the data obtained from the patient group (n=48) and the age and sex related control (n=48) was analyzed using the t-test. A p value < 0.001 with confidence limit 95% was defined as statistically significant. Numerical data are presented as mean±S.D. Microsoft Excel and WinStat were used for statistical analysis.

RESULTS AND DISCUSSIONS

1. Demographic characteristics:

The total number of females and males in both the control and patient groups were 13 (27.1%) and 35 (72.9%) respectively. The average age of the patients and control group were 59.1±10.9 and 58.5±10.7 respectively. Smokers and alcohol drinkers were 14.6% and 2.1% in patient group and there were no smokers and alcohol drinkers in controls. The basic diet was an Indian diet and 54.2% of the patients were non-vegetarians and 45.8% were vegetarians. In the control group 62.5% were non-vegetarians and 37.5% were vegetarians. In Table 1 the occupational characteristics of the patient group are presented.

Table 1
Occupational characteristics of the patient group

OCCUPATION	N	%
Farmers	5	10.5
Tannery/Chemical Industry workers	4	8.3
Business/Managerial Position/Teaching	8	16.6
Electrician	1	2.1
Manual Labour	2	4.2
Retired/Housewives	28	58.3

2. Clinical characteristics:

In the Echo findings of the patients it was observed that all patients had an ejection fraction (EF) ≤ 40%, with a mean value of 35.15±4.12. The fractional shortening (FS) was < 25 and the mean was = 18.7±3.7. 66.67% of the patients had Mitral regurgitation and 39.6% had tricuspid regurgitation.

3. Conventional CA analysis:

The frequency of the chromosomal aberration in patients and controls are given in Table 2.
The chromosomal aberration frequency per cell in patients was 0.092±0.04 which is significantly higher than in controls (0.005±0.0085) ($p<0.001$). Table 3 shows the mean CA in patients according to their dietary pattern. However, no significant difference is seen in aberrations due to the diet ($p> 0.001$). The frequency distribution of CA in the patient with their corresponding clinical data is presented in

Table 4. However, a correlation between the severity of the disease and the frequency of chromosomal aberration cannot be denoted. Figure 1 shows the mean CA obtained for patients when grouped according to their respective occupations; to account for occupational exposure to mutagens and formation of CA
.

Table 2
The frequency of CAs in PBLs of DCM patients using the conventional cytogenetic technique

	Patient (n=48)	Control (n=48)
Total number of metaphases analyzed	2400	2400
Total number of chromosomal aberrations scored (chromatid breaks+ dicentrics+ chromosome breaks respectively)	221+4+2 = 227	11+0+0 = 11
Mean frequency of chromosomal aberrations per cell	0.092±0.04	0.005±0.0085

Table 3
Mean CA according to dietary pattern of the patient group

DIETARY PATTERN	MEAN CHROMOSOMAL ABERRATIONS
Non-vegetarian (N=26, 54.2%)	5.3±1.9
Vegetarian (N=22, 45.8%)	4.1±1.5

Table 4
Frequency distribution of CA and clinical characteristics of the patients

NO. OF CHROMOSOMAL ABERRATIONS	NO. OF PATIENTS	AVERAGE EJECTION FRACTION	AVERAGE FRACTIONAL SHORTENING
2	3	29.33	14.7
3	12	35.2	20
4	11	35.9	19.4
5	8	34.87	17.75
6	3	38	19.3
7	6	34.6	17.86
8	2	38	19.5
9	2	35.5	19

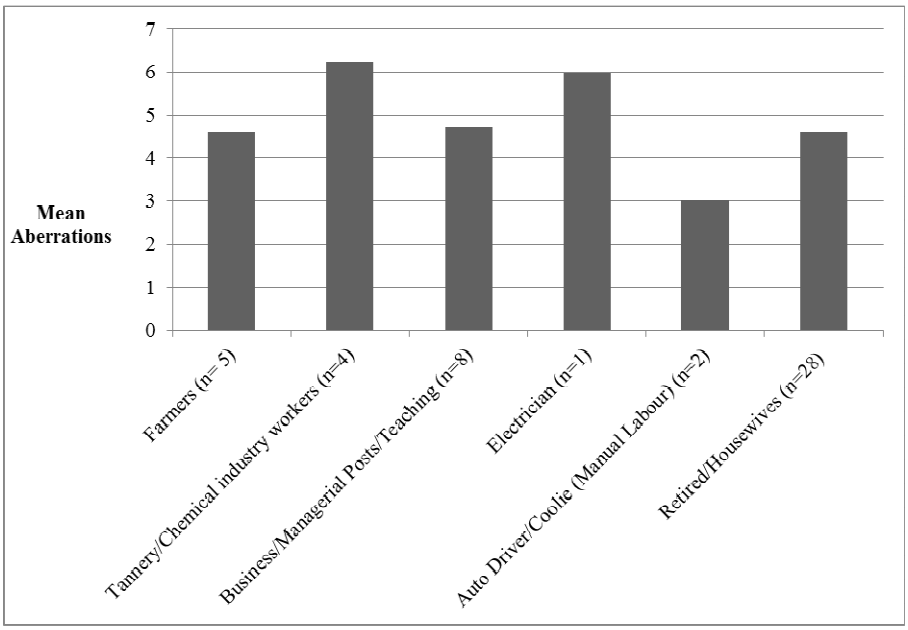

Figure 1
Frequency distribution of CA according to occupation of the patient group

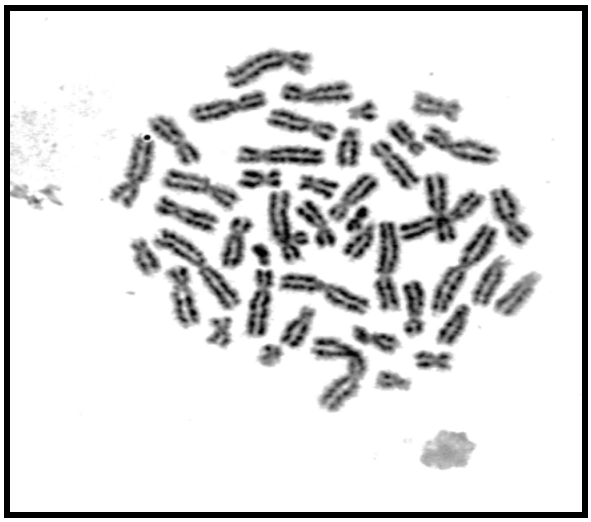

Figure 2
Normal Metaphase

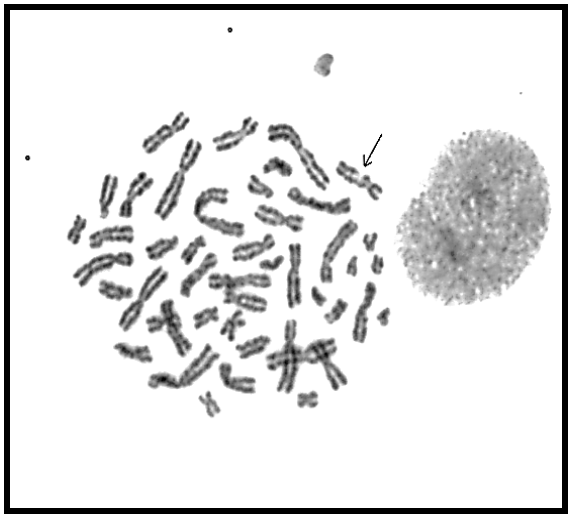

Figure 3
Metaphase with chromatid aberration

Main causes of death in the global population are cardiovascular diseases and cancer and it is hypothesized that these chronic degenerative diseases share several risk factors, such as oxidative stress and DNA damage. Although the role of DNA damage and mutation in carcinogenesis is well established[6] that one involved in vascular disease is less known. This study is the first of its kind reported, documenting the extent of DNA damage in dilated cardiomyopathy patients.

In this present study, by applying conventional cytogenetic analysis technique it was possible to bring out the high frequency of DNA damage expressed in various types of chromosomal aberrations (0.092±0.04).

The potential effect of occupational exposure on the increased CA frequency in DCM was estimated. As reported by Bonassi et al[7], in this study also it was observed that occupational exposure is not a confounder or a modifier of the association between CAs and DCM. Furthermore, as shown by the results, dietary pattern of patients did not have any effect on the increased frequencies of CA.

Although this study confirms the increased frequency of CAs in peripheral lymphocytes as a reliable early predictor of DCM, some limitations of this study have to be considered such as; the frequency of chromosome instability because the results are based on conventional cytogenetic analyses of unbanded metaphase preparations of PBLs. Future studies by fluorescent *in situ* hybridization would clarify the association between the frequency and type of chromosome damage in PBLs; secondly, administration of drugs to the patients may be responsible for the increased frequency of chromosomal aberrations. Therefore additional work with larger sample size has to be carried out to confirm this observation.

However this study provides an association between increased chromosomal aberration frequency and DCM, which could be used as a biomarker for validation.

ACKNOWLEDGEMENTS

We would like to thank the patients and their families for their cooperation. We also thank the management of VIT University for providing the facilities to carry out this study. The author Sneha Sitaraman is grateful to VIT University for providing the financial assistance during this tenure.

REFERENCES

1. Maron BJ, Towbin JA, Thiene G, Antzelevitch C, Corrado D, Arnett D, Moss AJ, Seidman CE and Young JB, Classification of the cardiomyopathies: an American Heart Association Scientific Statement from the Council on Clinical Cardiology, Heart Failure and Transplantation Committee; Quality of Care and Outcomes Research and Functional Genomics and Translational Biology Interdisciplinary Working Groups; and Council on Epidemiology and Prevention. Circ 113:1807-1816 (2006).
2. Botto N, Rizza A, Colombo MG, Mazzone AM, Manfredi S, Masetti S, Clerico A, Biagini A, Andreassi MG, Evidence for DNA damage in patients with coronary artery disease. Mutat Res 493*:* 23-30 (2001).
3. Murgia E, Maggini V, Barale R, Rossi AM, Micronuclei, genetic polymorphisms and cardiovascular disease mortality in a nested case-control study in Italy. Mutat Res 621:113-118 (2007).
4. Obe G, Pfeiffer P, Savage JRK, Johannes C, Goedecke W, Jeppesen P, Natarajan AT, Martinez-Lopez W, Folle GA, Drets ME, Chromosomal aberrations: formation, identification and distribution. Mutat Res 504:17-36 (2002).
5. Hungerford DA, Leucocytes cultured from small inocula of whole blood and the preparation of metaphase chromosome by treatment with hypotonic KCl. Stain Tech 40:333-338 (1965).
6. Tucker JD, Preston RJ, Chromosome aberrations, micronuclei, aneuploidy, sister chromatid exchanges and cancer risk assessment. Mutat Res 365*:*147-159 (1996).
7. Bonassi S, Hagmar L, Stromberg U, Montagud AH, Tinnerberg H, Forni A, Heikkila P, Wanders S, Wilhardt P, Hansteen IL, Knudsen LE and Norppa H, Chromosomal aberrations in lymphocytes predict human cancer independently of exposure to carcinogens. Cancer Res 60:1619-1625 (2000).