Altered DNA repair, oxidative stress and antioxidant status in coronary artery disease

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Coronary artery disease (CAD) is a multifactorial disease caused by the interplay of environmental risk factors with multiple predisposing genes. The present study was undertaken to evaluate the role of DNA repair efficiency and oxidative stress and antioxidant status in CAD patients. Malonaldehyde (MDA), which is an indicator of oxidative stress, and mean break per cell (b/c) values, which is an indicator of decreased DNA repair efficiency, were found to be significantly increased in patients compared to normal controls ($P<0.05$) whereas ascorbic acid and GSH were found to be lower among patients than the control group. It has been found that elevated oxidative stress decreased antioxidant level and decreased DNA repair efficiency can contribute to the development of CAD. This study also showed that high MDA, low ascorbic acid and GSH were significantly associated with high b/c value.

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1. Introduction

The global load of cardiovascular diseases (CVD) is rapidly amplifying, predominantly due to a sharp climb in the prevalence and incidence of the same in the developing countries. India, a developing nation, is experiencing the same stage and is now at the centre of a coronary artery disease (CAD) epidemic (Reddy 2004). Increased genetic propensity and increasing prevalence of cardiovascular risk factors are the reasons postulated for increased prevalence and higher severity and extent of CAD in Indians (Kasliwal *et al.* 2006). Conventional risk factors like hypertension, hyperlipidemia, diabetes mellitus, family history and smoking account for only about 50% of the total risk of CAD. This suggests that the major CAD risk factors are yet to be identified. Over the last decades, it has become significantly evident that oxidative stress presents a significant role in the pathogenesis of coronary atherosclerosis and its complications. Reactive oxygen species are the most likely agents inducing DNA damage in atherosclerosis (Mahmoudi *et al.* 2006). ROS generally exist in all aerobic cells in balance with biochemical antioxidants (Patri *et al.* 2009). Recently, increased oxidative stress and impaired antioxidant defence have been suggested as contributory factors for initiation and progression of complications in coronary artery diseases (Akila *et al.* 2007).

DNA damage has been found as an emerging risk factor to play an important role in atherosclerosis and coronary artery disease (Botto *et al.* 2002) and is caused by multiple endogenous and exogenous factors such as oxidative stress, age, smoking, hypertension, hyperlipidemia and diabetes mellitus (Andreassi and Botto 2003). Such damages, if left unrepaired, can cause mutations, which can lead to disease. To prevent accumulation of mutations and the production of altered proteins, cells deploy an arsenal of repair mechanisms to excise and replace defective nucleotides, reconnect

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Table 1. Distribution of MDA ascorbic acid, GSH and mean b/c value of subjects

Variables	Test (n=112)	Control (n=50)	*P*
MDA(nmol/mL)	1.46±0.68	0.81±0.2	0.000
Ascorbic acid (mg/dL)	1.09±0.62	2.88±0.9	0.000
GSH (mg/dL)	12.39±4.36	22.87±5.97	0.000
Mean b/c value	0.8020±0.069	0.6998±0.05	0.000

Values are mean±SD.

broken strands, and patch up other kinds of damage. Any alteration in DNA repair may increase the risk for CAD by influencing an individual's susceptibility to environmental mutagens and reactive cellular metabolites. A means of repairing DNA damage is vital to maintaining the integrity of the genetic blueprint (Kurthkoti *et al.* 2008).

So far, many studies have been conducted to evaluate the role of somatic DNA damage in CAD. No systematic studies were conducted to evaluate the role of DNA repair efficiency and to correlate it with oxidative stress and antioxidant status in CAD patients. Hence, the present study was undertaken to evaluate such factors so as to use its role in better diagnosis and prognosis of the disease.

2. Materials and methods

One-hundred-and-twelve clinically proven CAD patients referred from Pushpagiri Heart Institute, and fifty healthy age- and sex-matched controls were included in this study. Six milliliters of venous blood was collected aseptically from all the subjects after getting the informed consent. Two milliliters of blood was transferred into heparinized vacutainers and used for peripheral lymphocyte culture and *in vitro* mutagen sensitivity assay for determining DNA repair efficiency. Remaining 4 mL of blood was allowed to clot, serum separated and used for the estimation of malondialdehyde (MDA), an important marker of oxidative stress and for ascorbic acid and GSH, which are considered as the important parameters to assess the antioxidant status.

DNA repair efficiency was assessed by *in vitro* mutagen sensitivity analysis by determining the mean break per cell (b/c) value in lymphocytes exposed to mutagen in *in vitro* culture. Peripheral blood lymphocyte micro-culture was performed as described by Moorhead *et al.* (1960). Bleomycin [Sigma] (mutagen) was added to induce chromosomal breakage, chromatid breaks were scored and the mean number of breaks/cell (b/c) was calculated according to the method of Hsu *et al.* (1985). MDA estimation is based on the reaction of MDA with thiobarbituric acid (TBA), producing a MDA-TBA_2 adduct according to a modified version of Satoh's (1978) and Yagi's (1984) methods. Ascorbic acid was estimated according to Varley (2002) using DTC reagent (2,4-dinitrophenyl hydrazine-thiourea-CuSO). Glutathione (GSH) was estimated according to Beutler and Kelley (1963) in which 5, 5′-dithiobis (2-nitrobenzoic acid) (DTNB) and GSH react to generate a yellow-coloured product 2-nitro-5-thiobenzoic acid and glutathione disulfide (GSSG).

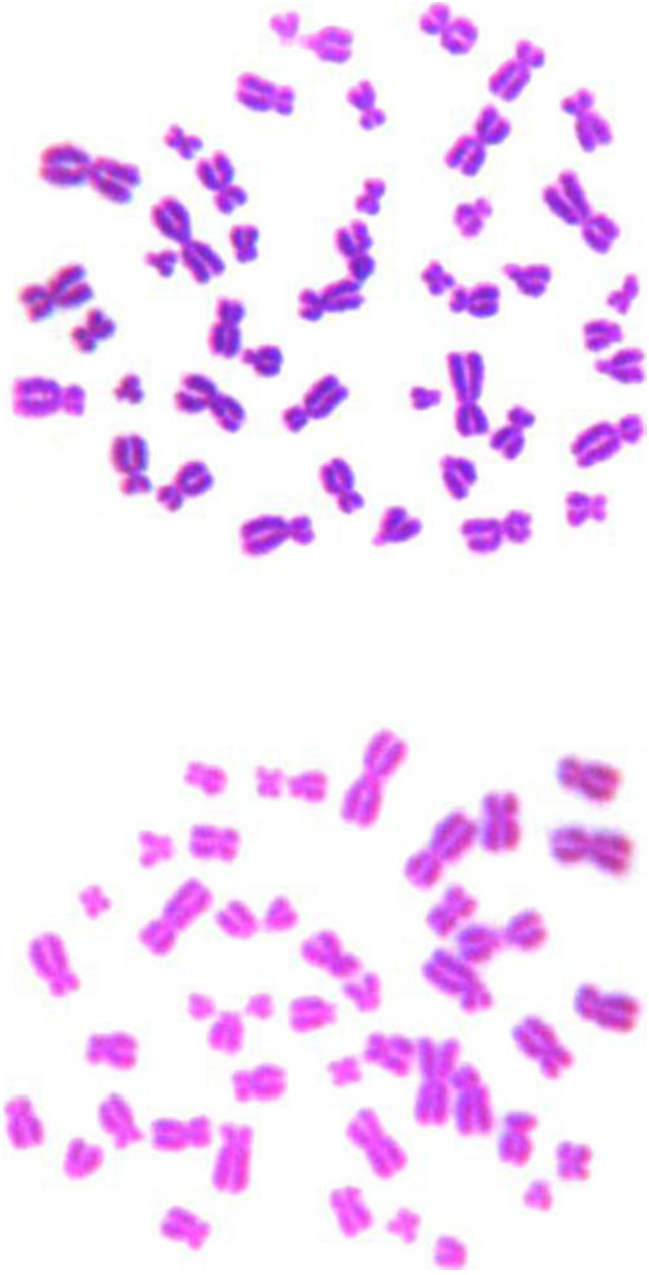

Figure 1. Geimsa-stained metaphases without chromatid breaks.

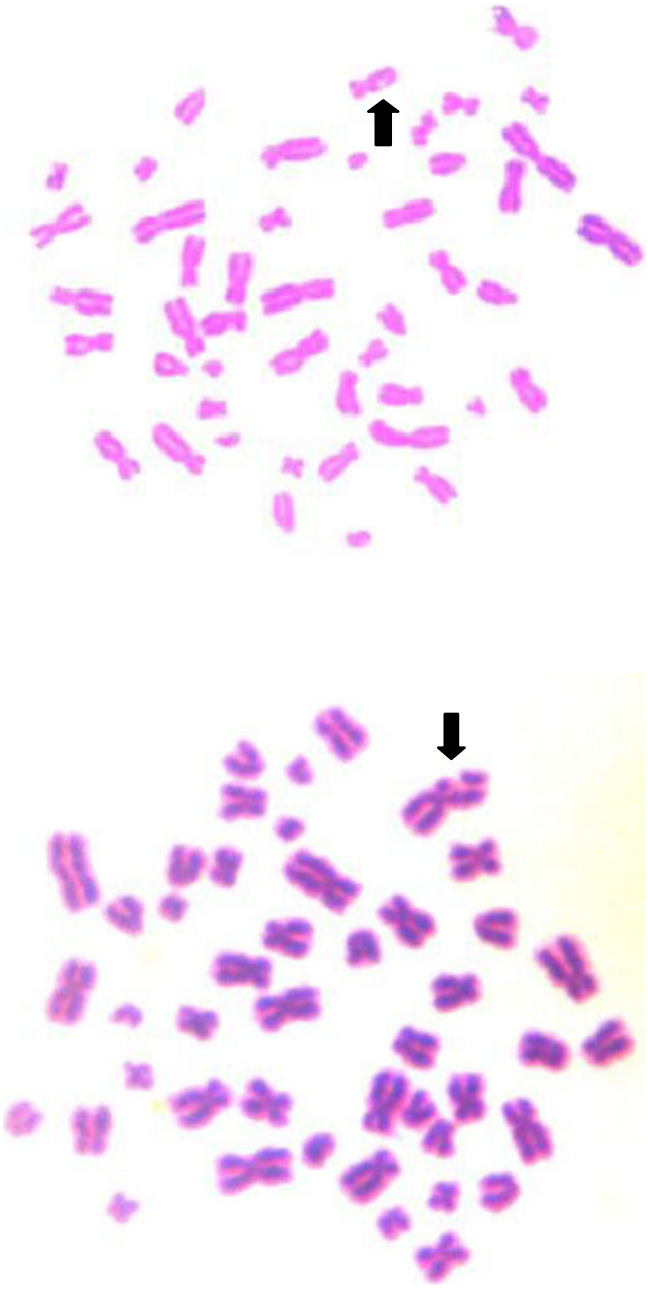

Figure 2. Metaphases showing chromatid breaks.

2.1 *Statistical analysis*

Results were expressed as mean±SD. 't' test, chi-square test and logistic regression analysis were performed for data analysis. The result was presented as odds ratio (OR) with 95% confidence intervals.

3. Results

The distribution of MDA, ascorbic acid, GSH and the mean breaks per cell values is given in table 1. MDA and the mean break per cell values were significantly higher, whereas ascorbic acid and GSH were found to be lower among test group than the control group ($P<0.05$). Figure 1 shows the images of Geimsa-stained metaphases without chromatid breaks, and figure 2 shows the images of Geimsa-stained metaphases with chromatid breaks.

Statistical data are given in table 2. High MDA, low ascorbic acid and GSH are found to be risk factors for CAD. Subjects having MDA value ≥1 nmol/mL have 8.20 times increased chance of developing CAD than subjects with MDA <1 nmol/mL. (odds ratio=8.20; confidence interval= 3.617–18.590). Subjects having ascorbic acid <1 mg/dL have 49.00 times increased chance of developing CAD than subjects having ≥1 mg/dL. Subjects with GSH <20 mg/dL have 97.7 times increased chance of developing CAD than subjects with GSH≥20 mg/dL. Increased mean b/c values, which is an indicator of decreased DNA repair pro-efficiency, was found to be significantly associated with CAD. Mean break per cell (b/c) value <0.8 is considered as normal, ≥0.8 b/c was considered sensitive, and those ≥1.0 b/c were considered hypersensitive. Subjects with mean b/c values≥0.8000 have 21.667 times increased chance of developing CAD than subjects with mean b/c values <0.8000 (odds ratio=21.667; confidence interval=6.358–73.830). Logistic regression analysis for coronary artery disease was performed for MDA, ascorbic acid, GSH and mean b/c value, and the results are given in the table 3. The variable which was found to be significant in the logistic regression analysis was GSH.

Association between MDA, ascorbic acid and GSH among the test and control with high mean b/c value is done by chi-square test, and are given in table 4. High MDA (≥1 nmol/mL), low ascorbic acid (<1 mg/dL) and GSH

Table 2. Association between MDA, ascorbic acid, GSH and mean b/c value among the test and control with coronary artery disease

Variables	Odds ratio	Confidence interval	χ^2	*P*
MDA (≥1 nmol/mL)	8.200	3.617–18.590	29.623	0.000
Ascorbic acid (<1 mg/dL)	49.000	6.538–367.246	34.924	0.000
GSH (<20 mg/dL)	97.778	21.536–443.935	80.685	0.000
Mean b/c value (≥ 0.8000)	21.667	6.358–73.830	38.43	0.000

Table 3. Logistic regression analysis showing correlation between MDA, ascorbic acid, GSH and mean b/c value with coronary artery disease

Variables	B	S.E.	Wald	df	Sig.	Exp(B)
MDA	0.534	1.130	0.223	1	0.636	1.706
GSH	6.094	1.905	10.237	1	0.001	443.179
Ascorbic acid	6.011	4.656	1.667	1	0.197	408.004
Mean b/c value	1.534	1.234	1.546	1	0.214	4.638

Variable(s) entered on step 1: MDA, GSH, ascorbic acid and mean b/c value.

(<20 mg/dL) were found to be significantly associated with high b/c value (≥ 0.8000). Logistic regression analysis for high (sensitive) mean b/c value (≥0.8000) was performed for MDA, ascorbic acid and GSH, and the results ere given in the table 5. Low GSH level is found to be significantly correlated with high (sensitive) mean b/c value.

4. Discussion

Many studies have linked excess generation of reactive oxygen species (ROS) with cellular damage and atherogenesis. Malondialdehyde is a decomposition product of autooxidation of polyunsaturated fatty acids is used as an index of oxidative damage. Kaur *et al.* (2008) found that serum malondialdehyde levels were significantly raised in all subjects with CAD as compared to control group. The present study observed a significantly high level of MDA in CAD patients compared to the normal counter parts ($P<0.05$), which is in agreement with the above studies.

An increased mean b/c value, which is an indicator of decreased DNA repair efficiency, was found to be significantly associated with coronary artery disease. The results are in agreement with the pervious study by Bazo *et al*. (2009) in which they found that DNA repair [X-ray cross-complementing group 1 (XRCC1)] genes polymorphism might be linked with heightened risk for CAD.

GSH is the most abundant intracellular thiol-based antioxidant and plays a significant role in the cellular protection cascade against oxidative injury (Hsu *et al.* 2002). This study revealed a significant correlation between CAD and GSH level. The findings are in well agreement with the previous investigations of Kaur *et al.* (2008). The significantly decreased levels of GSH in this study could be secondary to increased oxidative stress. Ascorbic acid is a water-soluble antioxidant that acts as the body's primary defense against peroxyl radicals formed in the aqueous phase. Earlier studies demonstrate a significant decline in the levels of vitamin C in CAD patients as compared to controls (Marjani 2005; Shaikh and Suryakar 2009). In the present study significantly decreased levels of ascorbic acid were observed in patients ($P=0.000$). This is in good agreement with all the earlier results.

Table 4. Association between MDA, ascorbic acid GSH and among the test and control with mean b/c value

Variables	χ^2	df	*P*
MDA (≥1 nmol/mL)	6.488	1	0.011
Ascorbic acid (<1 mg/dL)	25.253	1	0.000
GSH (<20 mg/dL)	26.919	1	0.000

Table 5. Logistic regression analysis showing correlation between MDA, ascorbic acid, GSH and mean b/c value

Variables	B	S.E.	Wald	df	Sig.	Exp(B)
MDA	−0.191	0.474	0.162	1	0.687	0.826
Ascorbic acid	0.782	0.470	2.771	1	0.096	2.185
GSH	2.304	1.115	4.273	1	0.039	10.014

Variable(s) entered on step 1: Ascorbic acid, MDA and GSH.

Increased oxidative stress can lead to an imbalance between DNA repair systems and DNA damage, resulting in accumulated oxidative damage with age (Lili *et al.* 2006). GSH protects cells against DNA damages and mutations from free radicals (Valko *et al.* 2006). In adjunct, a recent study suggests a function of GSH in DNA repair (Pujari *et al.* 2009), highlighting its important role in DNA protection. In the present study, high MDA were significantly associated with high mean b/c value. Similarly, low GSH and low ascorbic acid were shown to be risk factors for high/sensitive (>0.8) mean b/c value. These observations are in well agreement with the findings of Pujari *et al.* (2009).

This study distinctly shows that there is an altered DNA repair efficiency, increased oxidative stress and low antioxidant status in patients with CAD. A decreased antioxidant status mainly low GSH level is significantly correlated with altered DNA repair efficiency. Altered DNA repair efficiency is an additional risk factor for the development of CAD. The lifestyle modifications will reduce the risk of CAD by reducing oxidative stress and improving DNA repair efficiency.

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