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Research Paper

The relationship of lipid peroxidation and antioxidant status to selected modifiable risk factors in coronary artery disease patients

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ABSTRACT

Background: Coronary artery disease (CAD) is found to be associated with a wide range of modifiable and non-modifiable risk factors.
Aim of the Study: To evaluate the relationship of lipid peroxidation and antioxidant status to selected modifiable risk factors in angiographically proven CAD patients.
Methods: 150 angiographically proven CAD patients were categorized into three, based on selected risk factors. Data was collected using proforma and from hospital records. Peroxidation and antioxidant levels in blood samples were assessed using standard procedures.
Results: In category, I, significantly higher level of lipid peroxidation and the lower enzymatic antioxidant level were observed in patients with diabetes, hypertension, and with both diabetes and hypertension, when compared with patients without these clinical characteristics ($p < 0.01$). Similar results obtained for patients following a non-vegetarian diet when compared with patients following a vegetarian diet (category II). In BMI based group (category III), patients with BMI>25kg/m2 showed a significant increase in peroxidation and low enzymatic and non-enzymatic antioxidant levels than those with normal BMI.
Conclusion: The study confirmed a strong association between selected modifiable risk factors, higher lipid peroxidation, and lower antioxidant levels in angiographically proven CAD patients. This provides leads in the management of cardiovascular events in CAD patients.

1. Introduction

Coronary artery disease (CAD) is the most prevalent disease with the highest global mortality rate. According to the World Health Organization, CAD accounted for 17.6 million deaths per year in 2016 and maybe expected to rise to 23.6 million by 2030 [1]. Lipid peroxidation is a free-radical mechanism that plays a significant role in cardiac dysfunction pathogenesis [2]. A healthy antioxidant status is therefore critical for human health, in particular, to reduce peroxides and prevent chronic diseases such as CAD.

Different environmental and genetic factors concurrently implicated in CAD [3]. Age, sex, race and family background are the non-modifiable risk factors for CAD whereas the modifiable factors include increased blood pressure, cholesterol levels, triglyceride levels, diabetes, alcohol intake, smoking, eating patterns, obesity, etc. [4]. Several studies on associating risk factors with CAD are available. Researchers have shown a consistent link of hypertension to coronary artery disease [5]. Diabetes mellitus (DM) was also reported to play a major role in the propensity to CAD [6]. Obesity is increasingly recognized as an epidemic and a modifiable risk factor for CAD [7]. Collecting evidence from a host of clinical trials and observational studies, researchers concluded that individual adopting a plant-based diet display 16–32% reduction in cardiovascular disease mortality risk.

Researchers suggested that the effects of risk factors on CAD may differ across ethnic groups [8,9]. The high prevalence of CAD in India may be due to increased genetic risks and predominance of cardiovascular risk factors. Among all Indian states, Kerala has the highest prevalence of coronary artery disease, 7.4% in rural areas and 11% in urban areas [10]. Our previous studies showed that CAD intensity is closely linked to increased lipid peroxidation and decreased antioxidant status when compared to normal healthy subjects [11,12]. Hence, the aim of this study was to examine the relationship of lipid peroxidation and

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antioxidant status of CAD patients with selected modifiable risk factors.

2. Materials and methods

2.1. The study protocol (Fig. 1)

2.1.1. Exclusion criteria

In this study, from 280 patients, 130 patients excluded based on exclusion criteria such as age, liver disease, kidney disease, cancer, infectious disease, and heart failure.

2.1.2. Inclusion criteria

After excluding 130 patients, 150 patients (99 men and 51 women between the ages of 30–65 years belonging to central Kerala, South India) angiographically proven by the cardiologists at Pushpagiri Heart Institute, Thiruvalla, Kerala, India were selected for the study.

2.1.3. Data collection and categorization of patients

The study protocol was approved by the Institutional Ethics Committee of Pushpagiri Institute of Medical Sciences and Research Centre, Thiruvalla, Kerala, India (Reg.No.PIMS & RC/E1/388A/'09). The work has been carried out following the Declaration of Helsinki for the experiments involving humans. Before collection of data and blood samples, participants were informed about the methodology and objectives of the study, and they gave written consent to participate in this study. Data regarding conventional risk factors, height, weight, physical and nutritional habits were recorded using a proforma. Body mass index (BMI) of the participants obtained as weight (in kg) divided by square height (in m^2). Clinical characteristics like diabetes mellitus and hypertension were obtained from clinical laboratory findings in hospital records.

For this study, the patients were grouped into **three** criteria:

Criteria I-based on clinical characteristics (diabetes mellitus, hypertension).

Group Z: Patients without diabetes mellitus and hypertension.
Group A: Patients with diabetes mellitus.
Group B: Patients with hypertension.
Group C: Patients having both hypertension and diabetes.
Criteria II-based on Nutritional Habits.
Group D: Patients following a vegetarian diet.
Group E: Patients following a non-vegetarian diet.
Criteria III-based on BMI.
Group F: Patients with normal BMI.
Group G: Patients with BMI >25 kg/m^2

2.2. Reagents

All reagents were purchased from Himedia (Mumbai, India) Sigma (St. Louis, MO, USA) and Merck (Darmstadt, Germany).

2.3. Sample collection and analysis

10 ml of peripheral blood was collected from each patient and transferred to appropriate vacutainer tubes for various biochemical analyses. The lipid peroxidation level was assessed by estimating the levels of malondialdehyde (MDA) by the thiobarbituric acid method [13]. The enzymatic antioxidant superoxide dismutase (SOD) was assessed by Kakkar et al. method [14] and the non-enzymatic antioxidant ascorbic acid (vitamin C) was determined by Roe and Kuether method [15].

2.4. Statistical analysis

Continuous variables are expressed as the mean ± standard deviation. Box plots are used to visualize the distribution of data and the line inside each box represents the median line, the boxes symbolize the 25th and the 75th percentiles, and the lines outside the boxes indicate the highest and lowest values. The Kolmogorov – Smirnov test was used to assess the normality of the data. On comparison of data with more than two continuous variables, the differences between them were analyzed by one-way ANOVA and Kruskal – Wallis test and for the data with two variables, analysis carried out by unpaired *t*-test and Mann-Whitney *U* test as appropriate. The values of $p < 0.01$ were considered statistically significant. The Statistical package Maxstat Lite 3.6, MS Excel 2016, and R Studio version 1.0.143 were used for effective analysis.

3. Results

3.1. Analysis of peroxidation and antioxidant status in CAD patients

3.1.1. Comparison of peroxidation and antioxidant levels of criteria I patients (Fig. 2)

To examine the difference in peroxidation and antioxidant levels, criteria I CAD patients were categorized into four Groups based on selected clinical characteristics; Group Z (without diabetes and hypertension, n = 22), Group A (with diabetes, n = 26), Group B (with hypertension, n = 48), and Group C (with diabetes and hypertension,

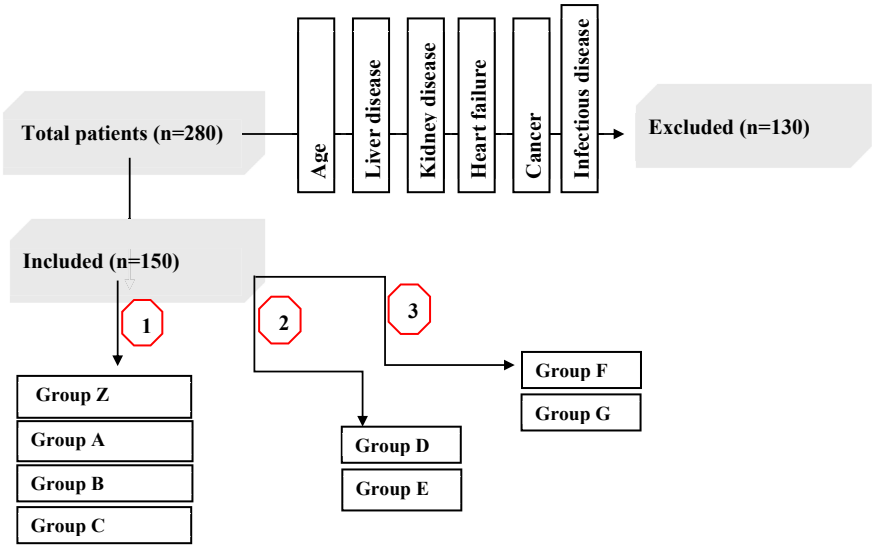


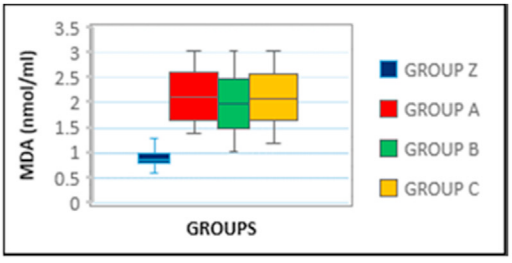
Fig. 1. The flow chart of protocol used in the study. 1. Grouping based on selected clinical characteristics. 2. Grouping based on nutrition. 3. Grouping based on BMI.

n = 54).

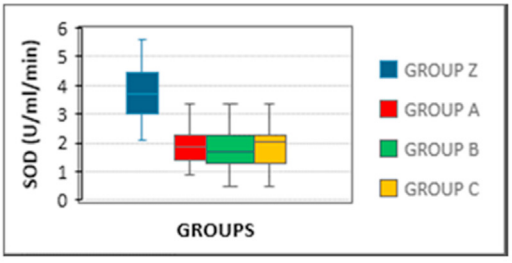
The comparative analysis of MDA levels in criteria I patients is shown in Fig. 2A. The box plot demonstrates that the variation of the inter-quartile range (IQR) in Group Z is minimum and high in Groups A, B, and C. The statistical analysis also shows that peroxidation (MDA) levels in Group A, B and C patients were significantly higher than in Group Z ($p < 0.01$) patients. (Group A: 2.146 [1.572to 2.721], Group B: 1.986 [1.457 to 2.515], Group C: 2.148 [1.598 to 2.698] versus Group Z: 0.905 [0.679 to 1.131].

Fig. 2B demonstrates the analysis of SOD level in Criteria I patients. The box plot shows a high interquartile range for Group Z, relative to Group A, Group B and Group C. Statistical analysis indicated that the level of SOD was significantly lower in patients with selected clinical characteristics than in Group Z ($p < 0.01$). (Group A: 1.853 [1.127 to 2.579], Group B: 1.747 [0.965 to 2.529], Group C: 1.864 [1.162 to 2.566] versus Group Z: 3.836 [2.671 to 5.001],

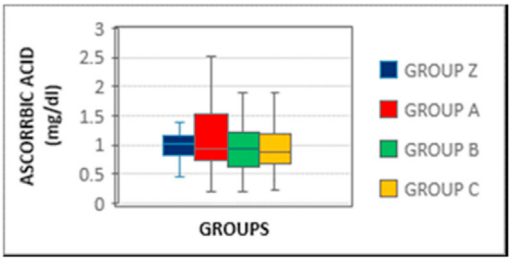
Fig. 2C depicts the analysis of ascorbic acid levels in Group Z patients relative to Group A, B and C. The IQR differences were minimal between groups Z, A, B and C. Furthermore, there was no statistical difference in ascorbic acid levels of groups with clinical characteristics from those in Group Z ($p > 0.01$). (Group A: 1.072 [0.492 to 1.652], Group B: 0.978 [0.520 to 1.436], Group C: 0.902 [0.523 to 1.281] versus Group Z: 1.103 [0.681 to 1.525].



A: Malondialdehyde levels of criteria I patients.($p<0.01$)



B: Superoxide dismutase level of criteria I patients. ($p<0.01$)



C: Ascorbic acid levels of criteria I patients. ($p>0.01$)

Fig. 2. Comparison of peroxidation and antioxidant levels of criteria I patients. **A:** Malondialdehyde levels of criteria I patients.($p < 0.01$). **B:** Superoxide dismutase level of criteria I patients. ($p < 0.01$). **C:** Ascorbic acid levels of criteria I patients. ($p > 0.01$). Group Z: Patients without diabetes and hypertension (n = 22). Group A: Patients with diabetes (n = 26). Group B: Patients with hypertension (n = 48). Group C: Patients having both diabetes and hypertension (n = 54).

3.1.2. *Comparison of peroxidation and antioxidant levels of criteria II patients (Fig. 3)*

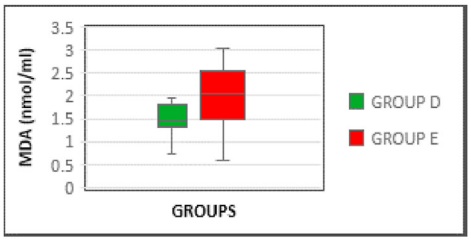
The criteria II patients were divided into Group D (vegetarian diet, n = 20), and Group E (non-vegetarian diet n = 130). The box plot (Fig. 3A) indicates that IQR of Group E is high in comparison to Group D. Statistical tests also confirmed a significantly higher peroxidation level in Group E than in Group D patients. ($p < 0.01$). (Group D: 1.492 [1.138 to 1.846] versus Group E: 1.979 [1.303 to 2.655].

From the figure (Fig. 3B), it is evident that the IQR of Group E is less in comparison with Group D. Statistical analysis proved that the SOD levels of Group E (non-vegetarian diet patients) were substantially less than those of Group D (vegetarian diet patients) ($p < 0.01$) Group D: 2.600 [1.888 to 3.312] versus Group E: 2.039 [0.931 to 3.147].

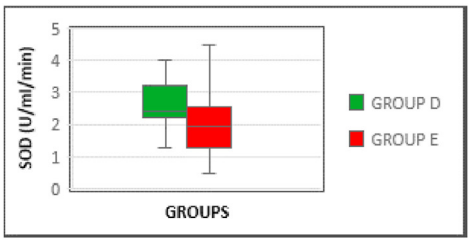
The ascorbic acid levels between two groups based on nutritional habits showed that IQR of Group D and Group E are the same. The statistical analysis did not indicate any significant variation of ascorbic acid levels between the two groups (Fig. 3C) ($p > 0.01$). Group D: 1.106 [0.597 to 1.615] versus Group E: 0.966 [0.524 to 1.408].

3.1.3. *Comparison of peroxidation and antioxidant levels of criteria III patients (Fig. 4)*

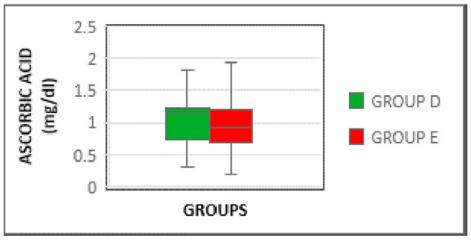
The patients in BMI based criteria were grouped into two; Group F (patients with normal BMI, n = 100) and Group G (patients with BMI



A: Malondialdehyde levels of criteria II patients.($p<0.01$)



B: Superoxide dismutase levels of criteria II patients.($p<0.01$)



C: Ascorbic acid levels of criteria II patients ($p >0.01$)

Fig. 3. Comparison of peroxidation and antioxidant levels of criteria II patients. **A:** Malondialdehyde levels of criteria II patients.($p < 0.01$). **B:** Superoxide dismutase levels of criteria II patients.($p < 0.01$). **C:** Ascorbic acid levels of criteria II patients ($p > 0.01$). Group D: Patients following Vegetarian diet (n = 20). Group E: Patients following non vegetarian diet (n = 130).

>25 kg/m^2 (n = 50).

Fig. 4A indicates that the IQR of Group F is less compared to Group G. The peroxidation level of patients in Group F was found to be significantly less than that of Group G patients. ($p < 0.01$). Group F: 1.789 [1.13 to 2.448] versus Group G: 2.162 [1.558 to 2.766].

SOD levels of BMI-based patients are shown in Fig. 4B. Statistical analysis confirmed that SOD levels in Group G with BMI> 25 kg/m^2 were significantly lower than in Group F patients with normal BMI ($p < 0.01$). Group F: 2.302 [1.126 to 3.478] versus Group G: 1.737 [1.01 to 2.464].

Ascorbic acid levels of BMI-based patients are evident in Fig. 4C. From the analysis, it is evident that ascorbic acid concentrations of patients with BMI >25 kg/m^2 (Group G) were significantly lower than normal BMI patients (Group F) ($p < 0.01$). Group F: 1.052 [0.618 to 1.486] versus Group G: 0.851 [0.387 to 1.315].

4. Discussion

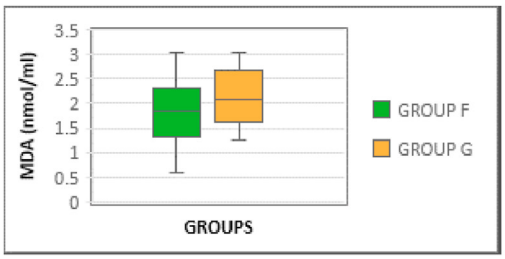
The Framingham Heart Study identified many risk factors associated with CAD. This study played a crucial role in raising health awareness of these risk factors, leading to a reduction in CAD morbidity and mortality [16]. Earlier reports suggest a clear linear association between the degree of CAD development and risk factors [17]. Zehra et al. (2006) reported an inverse association between MDA levels, antioxidant enzymes, and vitamins in CAD patients [18]. Only limited data are available on the relationship of lipid peroxidation and antioxidant levels in CAD patients with modifiable risk factors.

MDA, a widely used biomarker of lipid peroxidation reflects the oxidative stress of the biological systems [19]. In our study, MDA level of criteria I CAD patients showed a statistically significant increase in patients with clinical characteristics than those without the selected clinical characteristics. Consistent with our results, several reports showed increased peroxidation status in clinical conditions such as diabetes and hypertension [20,21]. Sivaprakash et al. (2006) reported the involvement of lipid peroxidation in CAD by significantly higher levels of MDA in diabetic patients [22].

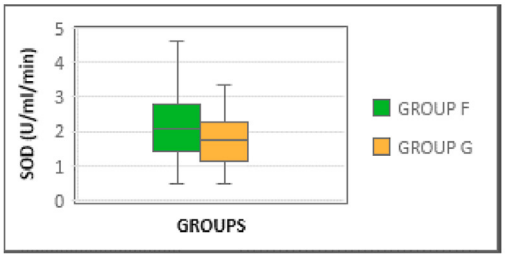
The enzymatic antioxidant (SOD) levels in criteria I patients indicated significantly low levels in patients with clinical characteristics, which suggests an important association with these risk factors. In patients with hypertension, decrease in antioxidant enzymes could be due to their inactivation by continuous exposure to peroxides and free radicals [20]. Jin-Song Zhao et al. (2018) reported a negative association between serum SOD activity and type 2 diabetes. The results suggest that decreased serum SOD activity was associated with poor glycemic control and the development of microvascular damage [23]. Reports on low serum SOD concentration in individuals with hypertension and diabetes suggests a deficiency in antioxidant protection mechanisms [24].

Earlier reports show that in a normal individual, insulin aids renal reabsorption of vitamin C, while hyperglycemia inhibits renal reabsorption of vitamin C [25]. Hypertensive individuals also reported having low plasma vitamin C levels [26]. In this study, analysis of non-enzymatic antioxidant, ascorbic acid levels in criteria I patients showed no substantial difference between the four groups. The probable reason for the results may be due to exclusion of patients with kidney disease, liver disease etc. Kenzo et al. (2005) reported that in type 2 diabetic patients, low levels of serum vitamin C were closely associated with concomitant renal dysfunction and low-grade inflammation [27].

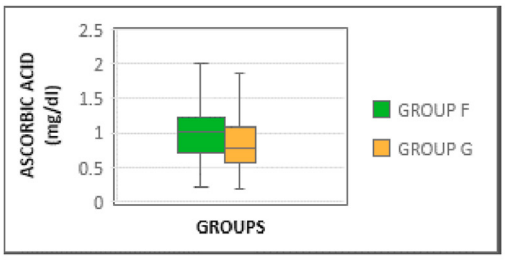
Our observations on criteria II patients, indicated a significantly higher level of lipid peroxidation in patients following the non-vegetarian diet (Group E) when compared to patients following a vegetarian diet (Group D). In accordance with our results, earlier reports also confirm that a non-vegetarian diet may cause atherosclerosis via macrophage activation whereas a plant-based diet low in fat, salt, sugar, etc. is associated with a lower incidence of CAD [28]. Vegetarians usually consume more fruits and vegetables than omnivores, so they have a lower intake of saturated fatty acids and increased intake of fibre-rich food and various kinds of antioxidants compared to those of non-vegetarian [29,30].



A: Malondialdehyde levels of criteria III patients.($p<0.01$)



B: Superoxide dismutase levels of criteria III patients. ($p<0.01$)



C: Ascorbic acid levels of criteria III patients.($p<0.01$)

Fig. 4. Comparison of peroxidation and antioxidant levels of a criteria III patients. **A**: Malondialdehyde levels of criteria III patients.($p < 0.01$). **B**: Superoxide dismutase levels of criteria III patients. ($p < 0.01$). **C:** Ascorbic acid levels of criteria III patients.($p < 0.01$). Group F: Patients with normal BMI. (n = 100). Group G: Patients high BMI > 25 kg/m^2 (n = 50).

In the case of the nutritional group, a statistically significant decrease in SOD levels was obtained for patients having a non-vegetarian diet (Group E) when compared with patients having a vegetarian diet (Group D). This indicates a strong relation to lower antioxidant levels and nutritional habits and is concomitant with earlier reports [29,30]. This observation point out the need to increase the antioxidant status thereby lowering oxidative stress in CAD patients.

During the study, ascorbic acid levels in Criteria II patients did not show any association with nutritional habits. This result may be due to possible effect of various factors affecting the antioxidant status. Earlier reports shows that ascorbic acid (vitamin C) status in humans is influenced by environmental, demographic, socioeconomic factors and health aspects [31].

According to previous reports, obesity is associated with increased production of free radicals or depleted antioxidant status [32]. Our study on criteria III patients also showed similar results. The lipid peroxidation level was high in patients with BMI >25 kg/m^2 (Group G) while low in the case of normal BMI patients (Group F) and the difference was statistically significant. Reports also show that the MDA level decreased markedly with weight loss [33].

In line with previous studies, our study on SOD and ascorbic acid levels also indicated lower levels with patients having BMI >25 kg/m^2 (Group G) when compared to normal BMI patients (Group F) [32]. The

results thus indicated an inverse association between antioxidant levels and BMI. Faienza et al. (2012) reported reduced antioxidant status in obese children [34].

4.1. Significance of the study

The present study highlights the possible association of modifiable risk factors with the peroxidation and antioxidant status of CAD patients. To the best of our knowledge, it is the only study from Central Kerala, India which pointed out the need for controlling the risk factors in angiographically proven CAD patients. Thus the study provides leads for prevention and management of future cardiovascular events in CAD patients.

4.2. Limitations of the study

In this study, one of the limitations is the sample size which was not very large and not uniform in the groups included in each criteria. From 280 angiographically proven CAD patients, only 130 patients were selected for the study due to defined exclusion criteria such as age, liver disease, kidney disease, heart failure, cancer and infectious diseases. Even though, we found significant associations during the study, a large sample size may provide better results. Another limitation is that it includes patients, only from Central Kerala, India.

5. Conclusion

The present study indicates a close association between higher peroxidation and lower antioxidant levels in CAD patients with selected modifiable risk factors. It also suggests that strict regulation of modifiable risk factors can be useful for primary and secondary CAD prevention. However, more investigation is needed to reveal the relevance of risk factors to CAD prevention and treatment.

Author contributions

Anoop Vijayan: Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft; Writing - review & editing. **Chithra V**: Conceptualization, Methodology, Formal analysis, Investigation; Writing - original draft; Writing - review & editing. **Sandhya C**: Conceptualization, Formal analysis, validation, Writing - original draft; Writing - review & editing. All authors have read and approved the final manuscript.

Ethics approval

The work was carried out following the Declaration of Helsinki for the experiments involving humans. The patients gave written informed consent to participate in this study. The study protocol was approved by the Institutional Ethics Committee of Pushpagiri Institute of Medical Sciences and Research Centre, Thiruvalla, Kerala, India Reg. No.PIMS & RC/E1/388A/'09.

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The statement of authorship

"The first author, second author and corresponding author takes responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation".

Declaration of competing interest

The authors declare no conflict of interest.

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