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Original Article

Oxidative stress and antioxidant status in cardiovascular diseases in population of western Nepal

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Abstract

Objectives: To observe if there is any connectivity between oxidative stress and cardiovascular diseases (CVDs).

Materials and methods: Patients suffering from different cardiovascular diseases (hypertension, ischemic heart disease, rheumatic heart disease) attending Manipal Teaching Hospital, Pokhara and strictly matched controls were selected for this study. Oxidative stress (OS) was measured by plasma thiobarbituric acid reacting substances (TBARS) where as antioxidant status was measured by estimating vitamin E, vitamin C and total antioxidant activity (TAA) in plasma.

Results: The mean level of TBARS, TAA, vitamin C and E were 2.20±0.43 nmol/ml, 547±98 μmol/l, 0.88±0.15 mg/dl and 0.75±0.20 mg/dl respectively in patients. The respective values in controls were 1.86±0.43 nmol/ml, 859±139 μmol/l, 0.94±0.15 mg/dl and 1.10±0.30 mg/dl. Although the OS seems to be raised in patients, is practically insufficient to oxidize biomolecules and induce CVDs. Despite vitamin C and E levels being well within normal limits, the TAA was significantly and considerably lower in patients. This is a highly interesting observation suggesting that dietary antioxidants other than these vitamins were preferentially consumed to control OS because procedure for TAA used in this study practically measures only total dietary antioxidants.

Conclusion: OS does not appear to be an etiological factor for the cardiovascular diseases; rather slightly raised OS in patients seems to be a consequence. Further the raised OS was not due to lower nutrient antioxidant (vit. C and vit. E) in the local population studied herein.

Key words: Oxidative stress, cardiovascular diseases, vitamin C, vitamin E, total antioxidant activity.

The world is poised for the tidal wave of CVDs. It is responsible for 10% of DALYs (disability-adjusted life years) lost in low and middle-income countries and 18% in high income countries.[1] The aetiology and pathophysiology of CVDs are complex, but the major risk factors include unhealthy life-styles and behaviours coupled with a multifactorial complex interaction between environment and genetic factors.[2] Growing evidence suggests that highly reactive oxygen derived free radicals (ROS) of endogenous or environmental origin play a cognitive role in the genesis and progression of various CVDs.[3,4] Normally these free radicals are effectively kept in check by the various levels of antioxidant defenses. Imbalance of this reaction either due to excess free radical formation or insufficient removal by antioxidants leads to oxidative stress (OS).[5] Established risk factors such as tobacco use (specially smoking), drinking , diet, pollution, exercises and metabolic abnormalities lead to the increased OS due to excess free radical activity [6] and that, these ROS can stimulate oxidation of low-density lipoprotein (LDL), cholesterol, cholesterol derived species, protein modifications which can lead to foam cell formation and atherosclerotic plaques.[7] It is therefore, logical to presume that antioxidants should help to prevent the CVDs. There is supportive evidence that vitamin C and E exert protective effect against CVDs by reducing OS,[8,9,10] though some doubts have very recently been raised.[11] Despite ROS and antioxidants being of central attention in CVDs, especially atherosclerosis, hypertension, myocardial infarction and stroke all over the world, to best of our knowledge, no work has been reported on the Nepali population so far.

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In this study we have estimated Thiobarbituric acid reacting substances (TBARS) as an indirect measurement of ROS production [5] and total antioxidant activity (TAA), vitamin E and vitamin C in the patients of hypertension, ischemic heart disease and rheumatic heart disease and matched controls.

Material and methods

This study included 20 already diagnosed CVD patients (Hypertension =6, Ischemic heart disease=7 and rheumatic heart disease=7) attending Manipal Teaching Hospital, Pokhara, Nepal, and the same number of normal healthy subjects, without any known disease. Six ml. of blood was collected from each subject by venipuncture with standard blood collection technique. Blood was transferred to EDTA (12mg) containing vial. Sample was mixed properly with the anticoagulant then transferred to the centrifuge tube. Samples were centrifuged for 10 minutes at 3000 RPM. The plasma was transferred to another labelled vial and analyzed promptly or stored in deep fridge for analysis within 24 hours. Plasma levels of vitamin C[12], vitamin E[13], total antioxidant activity (TAA)[14] and thiobarbituric acid reacting substances (TBARS)[15] were measured by standard methods. Statistical analysis was done by using computer based SPSS-11.0 version software programme. Student t- test and chi- square tests were applied wherever applicable to find out the level of significance and p value <0.05 was considered as the level of significance.

Results

The observations of this study are tabulated in the tables from 1 to 3.

Table 1: Biochemical parameters in patients and controls

	n	Age ±SD (yrs)	Vit. C mg/dl (μmol/l)	Vit.E mg/dl (μmol/l)	T AA μmol/l	TBARS nmol/ml
Case	20	52.15 ±17.20	0.88±0.15 (49.90± 11.30)	0.75±0.20[c] (15.80±4.22)	547±98[c]	2.20±0.43[b]
Control	20	48.20± 26.50	0.94±0.15 (53.37±8.50)	1.10±0.30 (23.20±6.33)	859±139	1.86±0.43

Note: a= p value < 0.05, b=p value< 0.01, c=p value< 0.001

Table 2: Biochemical parameters in males and females

	Sex	n	Age ±SD (yrs)	Vit.C mg/dl (μmol/l)	Vit.E mg/dl (μmol/l)	T AA (μmol/l)	TBARS (nmol/ml)
Case	Male	14	57.60 ±15.10	0.90±0.20 (51.10± 5.60)	0.80±0.20 (16.80±4.22)	528±88	2.20±0.38
	Female	6	39.30± 15.70	0.84±0.10 (47.69±5.60)	0.60±0.20 (12.66±4.22)	593±114	2.20±0.56
Control	Male	15	50.70 ±26.10	0.90±0.10 (51.10± 5.60)	1.10±0.30 (23.20±6.30)	831±139	1.82±0.48
	Female	5	40.80± 29.40	0.98±0.13 (55.64±7.38)	1.10±0.10 (23.20±2.10)	944±112	2.00±0.27

Table 3: Biochemical parameters in smokers and non-smokers

	Habit	n	Age ±SD (yrs)	Vit.C mg/dl (μmol/l)	Vit.E mg/dl (μmol/l)	T AA (μmol/l)	TBARS (nmol/ml)
Case	Smokers	8	56.37 ±8.83	0.84±0.10 (47.60± 5.60)	0.70±0.20 (14.70±4.22)	561±98	2.33±0.50
	Non-smokers	12	49.33± 29.95	0.90±0.20 (51.10±11.30)	0.70±0.30 (14.70±6.33)	538±102	2.10±0.37
Control	Smokers	9	49.33 ±20.95	0.86±0.10 (48.80± 5.66)	1.10±0.30 (23.20±6.30)	846±144	1.82±0.49
	Non-smokers	11	33.45± 24.48	1.10±0.10 (56.78±5.60)	1.10±0.20 (23.20±4.20)	870±141	1.90±0.40

Discussion

The role of free radicals through heightened OS, and averred role of antioxidants in CVDs is based on the premise that free radicals can injure arteries, can induce atherosclerosis by inducing fatty streaks resulting in atheroma by oxidation of LDL or possibly HDL also, can injure myocardium during reperfusion in MI and can cause hypertension by deregulating nitric oxide production and that antioxidants can prevent or deter most of these processes.[16,17,18] In the present study, the OS in CVD patients was mildly but significantly raised (2.20± 0.43 nmol/ml) compared to controls (1.86± 0.43 nmol/ml) (Table 1). The question arises whether the magnitude of this rise is cause of the disease or is just a consequence of it. In this regard, we would like to point out two types of observations; first, the intensity of pro-oxidant conditions required to oxidize lipoproteins (LDL and HDL) in-vitro is much higher, second, to obtain similar changes in cell culture studies, much higher pro-oxidant is required, therefore, the observations of these studies do not simulate to human and are not seen even in extreme cases of CVDs. As such we believe that this mild increase in OS is mere consequence. This is further supported by the fact that 3 groups of patients included herein did not show any significant variation from each other. It is further attested by the finding that IHD patients which were under far more stress than hypertensive patients had lower TBARS level (2.11± 0.49 nmol/ml) than hypertension group (2.39± 0.31 nmol/ml). In recent year several studies have shown that OS is more often a consequence of redox changes during disease process. [19, 20]

Smoking is established harmful factor to human health .[21] Several workers have claimed that the major damage is done by free radicals present in smoke, as a single puff is loaded with $1X10^{14-15}$ free radicals.[22] These observations are also mainly based on experimental studies and human observations are still inconclusive. Our data on OS do not show any difference between smokers and non-smokers in both case and control groups (Table 3).

As regard to antioxidants, the observations are still more important. No significant difference was observed in vitamin C status between patients and controls. Vitamin E status in patients was slightly but significantly lower than controls; nevertheless the level was sufficient enough to exert its optimal activity. As such the vitamin E deficiency is not an influencing factor for raised OS. On the other hand, the TAA which is a far better index than individual antioxidants was down by 36.4% in patients (Table 1). The FRAP procedure has advantage that it measures the TAA contributed by diet, because it does not measure the thiol group (reduced glutathione, proteins, -SH groups and other -SH containing compounds) which comprises >90% of non-enzymatic antioxidant activity.[14] This low TAA could be due to self imposed dietary precautions by patients or due to increased consumption of dietary antioxidants other than nutrient antioxidants. This is an interesting and novel observation in Nepali population as TAA activity has been seen to be normal in cancer and CVD in many other populations.

To conclude, taking all the data together it appears that raised OS in CVDs is more likely a consequence than cause of it; that this raised OS is not due to deficiency of antioxidant vitamins C and E. Lower TAA is a characteristic finding and should be taken up in future studies for seeking explanation.

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