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Effect of antioxidant serum levels of myocardial ischemia markers in patients with ischemic heart disease after treadmill exercise testing

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Heavy physical exercise increases oxygen consumption, and potentially initiates formation of reactive oxygen species (ROS), which leads to oxidative stress and cellular damage if not properly counteracted. The increase in malondialdehyde (MDA), released after intracoronary platelet aggregation might be a biochemical marker of coronary artery disease (CAD). Meanwhile, oxygen-derived free radicals after temporary coronary occlusion causes myocardial stunning.[1] Exercise leads to an increase in metabolic rate, production of ROS, and compromised antioxidant defense systems. The development of treadmill exercise testing, has allowed early and better evaluation of electrocardiogram (ECG) results, during and after exercise testing for the detection of ischemic changes. We undertook the present study to determine whether the plasma antioxidant status could help in confirming the diagnosis of CADs in borderline cases of treadmill exercise testing results. Also, whether changes in the parameters involved could help in the early detection and possible prevention of CADs especially in people with a family history.

We selected 62 subjects (42 patients and 20 control). We positively diagnosed all patients with CAD. We instructed all not to take any cholesterol lowering drugs and stop taking β-blockers or calcium channel blockers 3 days before doing the exercise test. We applied the Bruce protocol, starting with an initial work rate low enough for the least able subject. Progression continued until we reached the rate suitable for the most vigorous subject. We used stages of 3 minutes duration, permitting 5 different exercise intensity levels, which may be spaced more closely in terms of work rate and thus, may be more precise in measuring maximal functional capacity.[2] We terminated the test on any evidence that further exercise might be harmful to the subject. The exercise endurance in males was approximately 11.5 minutes and in females 7.6 minutes. We only included patients with true positive exercise results in this study. After the return of all parameters to recovery, we asked the patient to hyperventilate for 2 minutes, and we took a 12 lead ECG, which may reproduce the ST-depression in suspicious positive cases.

We took blood samples immediately before, and half an hour after finishing the exercise test. The biochemical tests included: lipid profile total cholesterol (TC), triglycerides (TG), high density lipoproteins (HDL), low density lipoproteins (LDL) and very low density lipoproteins (VLDL), serum very low density lipoproteins cholesterol (VLDL-C), and low density lipoproteins cholesterol (LDL-C) was calculated by Friedewald formula. The formula is only valid at serum triglyceride concentration of less than 400 mg/100 ml.

We measured lipid peroxidation in the form of MDA, a secondary product of lipid peroxidation. Its measurement is based on the colorimetric reaction with thiobarbituric acid. We measured uric acid, and albumin as antioxidants and the last parameter was creatine kinase. The heart rate progressively increases with every stage as maximal aerobic exercise capacity is reached, then it reaches a plateau like the oxygen consumption curve. There was a progressive increase in systolic blood pressure with increasing exercise intensity and very little change in diastolic pressure. At peak exercise it ranged from 162-216 mm Hg, while the diastolic may fall slightly in younger age groups, however, in middle-aged people this may rise not to exceed 10 mm Hg. A pathological fall in systolic blood pressure is highly specific for severe CAD. Failure of systolic blood pressure to rise reflects an inadequate elevation of cardiac output especially in left main stem disease, or equivalent coronary disease, and in 3 vessels disease.

The results of the positive exercise test revealed that the ECG changes of ST-depression will be found mainly in the bipolar leads. Only 2 cases out of 55 positive cases showed changes in the standard leads (3.6%). There was a significant rise of mean albumin level in the post-exercise samples of the control and patient groups **(Table 1).**

The pre-exercise samples of the patients group showed lower levels of albumin than the control group. The percentage rise in the control group more than the patient group. Albumin is considered a sacrificial antioxidant,[3] and the hypervolemia resulted after exercise account for the increased

level of albumin post exercise in the control and patient groups. The lower percentage of increase in the patients group is due to overuse as an antioxidant.

There was a significant increase in the mean level of uric acid in post-exercise samples of the control and patients groups (**Table 1)**. The percentage increase in the control group (17.6%) is more than double the increase in the patients group (6.6%). This is due to uric acid is considered an antioxidant, and it scavenges radicals and inhibits lipid peroxidation. This suggests a defect in the antioxidant status of the patient group, and high lipid peroxidation levels.[3] A significant increase in the mean level of creatine kinase was found to exist in the post-exercise of control and patient group. The percentage increase in the patients group was 55% while in the control group was only 49%. The rise could be more pronounced after days, or even weeks.[4]

Oxygen free radicals generated in vivo react with polyunsaturated fatty acids and form new radicals (peroxyradicals), which initiates a chain reaction of lipid peroxidation in the presence of oxygen. Therefore, MDA level is an indicator of free radicals generated in the human body. The level of mean MDA showed a significant increase in the post-exercise samples of both control group and the patients group ($p<0.05$) **(Table 1)**. The rise in the female patients (19%) is more than the male patients (16%), which could be due to wider deprivation of antioxidants in the female group of patients. The pre-exercise values show higher levels in the patients group (ischemic heart disease) than in the control group.

From the previous speculation and the present observation, it might be postulated that the high MDA level in ischemic heart disease cases is attributed to the circulating fractions of membrane lipids peroxidation products in addition to serum lipids. Decreased serum total antioxidant status is associated with increased MDA levels in coronary atherosclerosis.[5] A significant drop in the level of LDL in the post-exercise levels of the control group and in the post-exercise levels of the patient group as seen in **Table 1.** The percentage of decrease is more in the male patient group than in the others (-76%). Low-density lipoprotein is a major vehicle in distributing cholesterol from the liver to other tissues. The significant increase in the HDL level of post exercise level of the patient group is shown in **Table 1**. Testosterone increases with exercise, thus will decrease HDL formation. Significant correlation is found between HDL and MDA in the pre-exercise male control group. A significant drop in the level of VLDL in the post-exercise control group and in the post-exercise female and male patient groups is shown **Table 1.** Meanwhile, a significant decrease in the level of TG in the post-exercise samples of the control group and in the post-exercise level of the patient group can also be seen in **Table 1**. Both resistance training and moderate aerobic exercise increase serum testosterone levels of untrained subjects after 15-20 minutes. Testosterone will increase the formation of HDL, in males and in females, which will increase the metabolism of TG. With the utilization by myocardial cells for energy, this will account for the decrease in level of TG in the post-exercise blood samples. A significant decrease in the level of TC in the post-exercise control group, and in the post-exercise patient group is seen in **Table 1**.

All the cholesterol circulating in the blood is contained in lipoproteins. Control of body levels depends on the rate of excretion in the bile as cholesterol or bile salts in relation to the rate of

Table 1 - Lipid profile and some antioxidants measured in both groups (control and patients) pre and post exercise.

Levels	Control				Patients			
	Male		Female		Male		Female	
	pre	post	pre	post	pre	post	pre	post
Albumin g/dl	4.2	4.43	3.92	4.14	4.022	4.19	3.82	3.95
Uric acid mg/dl	5.66	6.66	4.11	4.79	5.51	5.88	3.89	4.15
Cholesterol mg/dl	197.5	156.9	179.9	156.8	207.05	184.41	209.65	186.5
Triglycerides mg/dl	104.7	85.3	80.4	66	163.95	139.95	138.5	118.1
HDL mg/dl	528	581	44.5	49	40.81	43.6	39	42.35
LDL mg/dl	105.76	81.74	119.32	94.6	199	112.9	142.45	120.681
VLDL mg/dl	21	17	16	13.2	32.8	28	27.7	23.6
MDA mmol/l	3.9	4.16	4.66	5.66	3.1	3.38	2.8	3.17

HDL - high density lipoproteins, LDL - low density lipoproteins, VLDL - very low density lipoproteins, MDA - malondialdehyde

synthesis in the liver from Acetyl-CoAm, which is regulated by feed back inhibition on reductase hydroxyl -methyl - glutary -CoA (HMGCoA) reductase by excess cholesterol. A high cholesterol diet causes decreased synthesis, and the excess is excreted in bile. The excreted bile salts are very efficiently reabsorbed more than dietary cholesterol.

Significant correlations have been found among the studied parameters in both groups studied, for example, between uric acid and albumin in the post-exercise male control group and in the pre and post-exercise control female group. There was a significant correlation between uric acid and albumin in the pre-exercised male patient group, and post-exercise female patients group. As well as between LDL and MDA in the post-exercise male control group and in the pre-exercise female patients' and VLDL and MDA in the post-exercise male control group. A significant correlation was found to exist between TG and MDA in the post-exercise female control group.

In conclusion, treadmill exercise testing is a very valuable aid in predicting and following cases of ischemic heart disease. The state of lipid peroxidation and antioxidants level could give a clue to the diagnosis of cases at risk, especially when combined with treadmill exercise testing, and may influence the prognosis and prevention of CAD.

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Proliferating cell nuclear antigen index and nm23 expression in osteosarcoma in relation to disease-free survival and tumor grade

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Osteosarcoma cases that have similar histological structure and same grade may have variable responses to the identical chemotherapy regimens, and show different prognosis. The current most common indicator of prognosis of an osteosarcoma patient is his response to neo-adjuvant chemotherapy. This is determined by measuring the amount of necrosis within the tumor mass following neo-adjuvant chemotherapy, which is a very difficult task.[1] Certain prognostic markers are needed for osteosarcoma patients. Both nm23 and proliferating cell nuclear antigen (PCNA) are the biologic markers those have been documented to have relationships with various tumors.[2-5] The nm23 is a tumor suppressor gene that is identified to be involved in tumor metastasis. There are many reports on nm23 expression in tumors; however, literary information is inadequate concerning nm23 expression and osteosarcoma together.[4] The PCNA, is a proliferation marker and can be detected by immunohistochemical methods in cells. It has been shown to relate with DNA polymerase delta sub-group and proliferation cannot occur in eucaryotic cells without it.[2,4,5] The purpose of this study was to investigate the PCNA and nm23 expression in osteosarcoma cells and their correlation with disease-free life period and tumor grade in search of new prognostic and predictive factors.

Formalin fixed and paraffin embedded tumor tissues of 51 patients were used in this study. Twenty-six of the cases (51%) were women, while 25 (49%) were men aged between 9-26 (median 16) years. Primary localizations were distal femur in 15, proximal tibia in 20, humerus in 11, proximal femur in 4, and radius in one patient. All patients' records were examined to determine the disease free survival. Specimens were confirmed as osteosarcoma and graded according to anaplasia, pleomorphism and nuclear hyperchromasia. Intravascular invasion for each specimen was also recorded **(Figure 1).** All patients were treated by neo-adjuvant chemotherapy, radical surgery, and adjuvant chemotherapy. Surgical treatments were amputations for 36, Enneking procedures for 11, resection arthroplasties for 3, and Van Nes procedure for one patient. The follow up period, free from metastasis or recurrence was defined for each patient as disease-free life