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Are Iron Deficiency Anaemic Patients Prone To Cardiovascular Disease?

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Abstract: The property of iron to get reversibly oxidized and reduced is essential for its metabolic functions. Severe iron deficiency can produce cardiovascular disease. Wide range of biochemical markers are implicated in the pathogenesis of anaemia like Serum Ferritin, Malondialdehyde and Homocysteine. The purpose of the study is to assess serum ferritin, homocysteine and plasma malondialdehyde levels in Iron deficiency anaemic patients and to find whether anaemic patients are prone to cardiovascular disease or not. A case control study was carried out at Lata Mangeshkar hospital, Nagpur in which serum malondialdehyde and homocysteine levels measured in 30 diagnosed patients of Iron deficiency anaemia in the age from 19 to 40 years were compared with healthy controls. Malondialdehyde was estimated by the method of Randox laboratory. Serum homocysteine was measured by using ELISA kits. The data was analysed for correlation between levels of homocysteine and malondialdehyde with serum ferritin in patients of Iron deficiency anaemia. Serum malondialdehyde levels in patients with Iron deficiency anaemia were higher i.e. 0.680$\pm$0.349 (ng/ml) than in controls which were 0.144$\pm$0.102 (ng/ml) with a statistically significant value of $p < 0.05$.Statistically, there was also a significant rise in homocysteine levels in patients with Iron deficiency anaemia i.e.76.105$\pm$16.836(ng/ml) as compared to 27.57+12.11 (ng/ml) in controls. An inverse relation exists between serum ferritin and markers of oxidative stress. Iron deficiency anaemic patients have elevated levels of malondialdehyde and homocysteine than the control group and both these parameters may be common mediators in the pathogenesis of accelerated atherosclerosis.

Keywords: Ferritin, Malondialdehyde, Homocysteine, Anaemia, Iron deficiency.

INTRODUCTION

Iron deficiency anaemia (IDA) is the most common form of nutritional anaemia in both the developed as well as the developing countries, prevalence greater among the infants, pregnant women and the poor. WHO believes that about 1/3rd of the world's population (> 2 billion people) is anaemic mostly due to iron deficiency [1]. In mild cases, patients remain asymptomatic. When the anaemia is moderate, dyspnoea and fatigue may occur, while severe anaemia can produce cardiovascular disease.

The central importance of iron in the pathophysiology of disease is derived from the ease with which iron is reversibly oxidized and reduced. This property, while essential for its metabolic functions, makes ionized form of iron potentially hazardous because of its ability to participate in the generation of powerful oxidant species such as hydroxyl radical [2].

Anemia promotes oxidative stress due to inadequate tissue oxygen supply which leads to an increase in the free radical production ultimately leading to very low level of circulating red blood cells. As a result, mobile free radical scavengers in the blood are decreased thus, not providing any protection to the tissues from reactive oxygen species (ROS) mediated damage [3]. These increased reactive oxygen species, especially hydrogen peroxide inhibit superoxide dismutase (SOD) activity which may contribute to free radical propagation.

Heme is synthesized in the cytoplasm of maturing erythroid cells and reticulocytes. Heme synthetase is a mitochondrial enzyme, which catalyzes the formation of heme by insertion of iron into

protoporphyrin IX [4]. Lack of iron, therefore prevents haemoglobin synthesis and decreased erythropoiesis. The red blood cells formed also have a decreased life span.

Just like other cells of the body, the cells in the heart including the endothelial cells and the cardiomyocytes are susceptible to this oxidative stress, ultimately affecting the cardiac functions. But this cardiomyopathy of iron deficiency anaemia may be completely reversible [5].

Serum ferritin is a biochemical marker used for diagnosis of IDA. Low levels are diagnostic of anaemia [6, 7]. Plasma Malondialdehyde (MDA) is the product of lipid peroxidation and is itself an indicator of free radical induced tissue damage [8]. Homocysteine is a common non-protein amino acid in our blood (normal levels are between 5-15 µmol/L. Hyperhomocysteinemia occurs when homocysteine levels in blood are above 15 µmol/L [9]. This has been claimed to be a significant risk factor for the development of a wide range of diseases, which is a strong indicator of the risk of future cardiovascular disease [6]. It degrades and inhibits the main structural components of the artery i.e. collagen, elastin and proteoglycans [10]. The evaluation of oxidative stress using these markers is receiving attention as they serve as early markers of oxidative stress in humans. No published studies have investigated correlations between homocysteine, MDA and the biochemical markers for IDA. So, numerous investigations need to be performed to determine relations between these.

OBJECTIVES

- To determine homocysteine levels in IDA patients and controls
- To determine MDA levels in IDA patients and controls
- To determine serum ferritin levels in IDA patients and controls
- To correlate serum ferritin levels with Homocysteine and MDA levels in both IDA patients and controls.

MATERIALS AND METHODS

This is a case control study for which approval was granted by the Institutional Ethics Committee, NKPSIMS & RC, Nagpur. The study enrolled 30 randomly selected subjects in the age group of 19-40 years diagnosed with iron deficiency anaemia, irrespective of their socio-economic status from Lata Mangeshkar hospital, Nagpur, Maharashtra and 30 controls not having iron deficiency anaemia. Only subjects diagnosed with iron deficiency anaemia with haemoglobin levels <10 gm% were included in the study.Patients with diagnosed cardiovascular disease, those who are suffering from any major chronic illnesses like diabetes, hypertension or any other endocrine disorder, patients on long term medications and pregnant women were excluded from the study. Any patient not willing to cooperate after initially signing the informed consent was allowed to withdraw from the study.

The outcome was assessed in terms of the role of homocysteine and Malondialdehyde in iron deficiency anaemic patients. 5 ml of venous blood sample was collected in a plain bulb from the patients as well as the controls. Complete blood count and Serum ferritin was done using ELISA kit. Malondialdehyde was estimated using the randox MDA estimation kit. Homocysteine was estimated using Qayee human HCY kit. The values obtained were analysed statistically to determine the relationship of MDA and Homocysteine with the biomarker for IDA i.e. serum ferritin in patients as well as controls.

RESULTS

The serum MDA levels in controls were 0.144±0.102 (ng/ml) and in patients with IDA were 0.680±0.349 (ng/ml) with a statistically significant value of $p < 0.05$.

Table-1 also shows statistically significant rise in Homocysteine levels patients with IDA (76.105±16.836 (ng/ml)) as compared to 27.57±12.11 (ng/ml) in controls.

Table-1: Showing the results of parameters to be correlated

PARAMETERS	CONTROLS (n-30) Mean+ SD	CASES (n-30) Mean+ SD
Hb (gm%)	12.093±2.067	7.826±1.169
Serum ferritin (ng/ml)	96.53±31.699	9.224±2.142
MDA (ng/ml)	0.144±0.102	0.680±0.349
Homocysteine (ng/ml)	27.57±12.11	76.105±16.836

P value for Hb – 0.003, for ferritin - <0.0000001, for MDA -<0.0000001 and for Homocysteine – <0.0000001

Table-2 Shows that Negative correlation (<0.3) were found between serum ferritin and MDA and homocysteine. Also negative correlation was found between MDA and homocysteine. Thus, an inverse relation exists between serum ferritin and markers of oxidative stress.

Table-2: Showing the correlation between the parameters

Correlation between		r-value	p-value
Serum ferritin	MDA	-0.0281	<0.0000001
	Homocysteine	-0.173	<0.0000001
MDA	Homocysteine	-0.164	<0.0000001

DISCUSSION

Normal physiologic response to anaemia is a compensatory increase in cardiac output to maintain adequate oxygen supply. The increased cardiac output increases the blood volume, preload, heart rate, and stroke volume, along with a decrease in afterload. In more severe cases of anaemia, the increased blood volume contributes to signs of congestion with peripheral and pulmonary oedema.

Anemia promotes oxidative stress due to inadequate tissue oxygen supply which leads to an increase in the free radical production ultimately leading to very low level of circulating red blood cells. Till date, many cardiovascular risk factors have been identified that affect endothelial functioning and in turn mediate vascular disease and its complications. Even other factors which are now well known for the pathogenesis of CVD like high cholesterol and oxidized LDL level have been suggested to initiate free radical induced lipid peroxidation and contribute to the pathogenesis of atherosclerosis [10]. During lipid peroxidation, unstable hydro peroxides result from peroxyl radical-dependent chain reactions which involve unsaturated fatty acyl moieties and later break down to smaller and more stable products like MDA (a thiobarbituric acid reacting substance (TBARS)) which are considered to be oxidative stress markers [10].

Since CVD have been generally described as having high levels of oxidative stress so in our study, we found correlations between Serum ferritin and MDA levels and also between serum ferritin and homocysteine levels in cases.

MDA levels were found to be significantly elevated (p value - <0.0000001) in patients with IDA as compared to healthy controls. Our study is in accordance with other studies where higher MDA levels have been reported in patients with IDA. It indicated increased oxidative stress, which is in turn reflected by increased lipid peroxidation in the peripheral blood of patients with IDA. This leads to increased cellular damage due to oxidative stress. Membrane fluidity decreases and the activity of membrane bound enzymes and receptors changes. These products of lipid peroxidation are harmful to most of the body cells and are associated with the risk of developing atherosclerosis [11].

In another study conducted by Dhananjay V. Bhale *et al*., the serum concentration of MDA was found to be higher in pregnant women with IDA [12]. *NL Madhikarmi and KRS Murthy in their research on lipid peroxidation and antioxidant system in IDA patients* suggested that increased lipid peroxidation products and reduced antioxidants system boost the oxidative stress state, and deteriorate the condition of IDA patients [13].

Various authors have proposed that increased levels of homocysteine and lipid peroxidation also play a significant role in the genesis of atherosclerosis and heart disease. It has been suggested that high concentrations, via the formation of thiolactone, could be responsible for the production of oxygen free radicals and in turn increase in lipid peroxidation [9]. Therefore, it confers an independent risk of vascular disease similar to that of smoking or hyperlipidaemia.

In a study conducted by Mahmoud Mohammed Sirdah *et al*, statistically significant negative correlations were reported for serum homocysteine and serum ferritin [14]. Similarly in our study, Negative correlation (r = -0.173) was found between serum ferritin and homocysteine. Nirjala L. Madhikarmi and Kora R.S. Murthy have said that cardiac complication is one of the leading signs in many diseases and all of this is because of an increase in the reactive free radicals which create an imbalance thereby making macromolecules vulnerable to oxidative damage [13].

Since both MDA and Homocyteine act as independent risk factors for cardiomyopathies, patients with increased levels of both are most likely to be at a greater risk than with one risk factor alone because of additive effect of more oxidative stress which will produce early and quicker damage of the heart cells.

There is experimental evidence suggesting that iron supplementation in IDA patients may activate molecular pathways protecting the heart and preventing myocardial remodelling.

Unfortunately there are not many published studies which have investigated these correlations, so there is need for large scale investigations to be performed to evaluate these.

CONCLUSION

Cardiovascular disease often goes unrecognized in patients with IDA; therefore, it is essential to carefully assess all patients shortly after the diagnosis of IDA is made. IDA patients have elevated levels of MDA and homocysteine than the control group and both these parameters are common mediator in the pathogenesis of accelerated atherosclerosis. Thus, we conclude that parameters of oxidative stress and homocysteine can help in assessment of cardiovascular status and improvement of health status in IDA patients.

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