

Original Research Article

Study of oxidative stress biomarkers in obese children

Avni Kanji Fariya, Bina F. Dias*

Department of Biochemistry, Lokmanya Tilak Municipal Medical College, Sion, Mumbai, Maharashtra, India

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***Correspondence:**

Dr. Bina F. Dias,

E-mail: binadias29@yahoo.in

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ABSTRACT

Background: The objective is to study the oxidative stress in obese and non-obese children by assessing the biomarkers of lipid peroxidation and antioxidant status, Malondialdehyde (MDA) and Ascorbic acid (vitamin C) respectively. Childhood obesity is a growing global epidemic that requires attention due to the burden placed on the healthcare system for children and adults. Consumption of fatty foods and a high sugar, fat diet, and no exercise qualify as the main reasons for obesity among children and adults. Childhood obesity is connected with an increased risk of various diseases such as diabetes, cardiovascular, stroke, certain types of cancer later in life, social problems and depression among youths. Obesity is also characterized by chronic low grade inflammation with permanently increased oxidative stress (OS). Over-expression of oxidative stress damages cellular structures together with under-production of anti-oxidant mechanisms, leading to the development of obesity-related complications.

Methods: The study involved 25 obese children for Vitamin C, 20 obese children for Malondialdehyde (MDA) and 18 non obese children for both Vitamin C and MDA in the age group of 5-14 years, without any complications. This study was conducted at L.T.M.M College. Plasma Ascorbic Acid was estimated colorimetrically by using 2,6-dichlorophenol indophenol dye and similarly Malondialdehyde was estimated colorimetrically by MDA-TBA colored complex.

Results: The study showed significantly higher values of MDA and lower level of Vitamin C in obese children as compared with non-obese children.

Conclusions: The levels of lipid peroxidation marker Malondialdehyde (MDA) is higher and level of antioxidant marker Ascorbic Acid (Vitamin C) is lower in obese children as compared with non-obese children. Thereby increasing oxidative stress and hence the oxidative damage to cells.

Keywords: BMI, Oxidative stress, Obesity, ROS, Vitamin C

INTRODUCTION

A major internal threat to the cellular homeostasis of aerobic organism arises from free radical intermediates and the by-product generated from oxygen metabolism. The paradox of aerobic life is that aerobic organisms cannot exist without oxygen and yet oxygen happens to be inherently dangerous to their existence. This is because ironically, these reactive oxygen species (ROS)

are derived from normal physiological and metabolic processes that are essential to the living cell.¹

Oxidative stress is an imbalance between tissue oxidants (free radicals or reactive oxygen species) and antioxidants and may be a unifying mechanism in the development of major obesity-related comorbidities such as cardiovascular disease (CVD) and diabetes. Oxidative stress is assessed by the index of lipid peroxidation and has been shown to be elevated in obese paediatrics.²

In present study malondialdehyde (MDA) and Ascorbic acid (vitamin C) are selected as biomarkers of oxidative stress. MDA is the principle end product of poly unsaturated fatty acid peroxidation. This aldehyde is highly toxic molecule. Ascorbic acid (vitamin C) is an antioxidant, it is hydrophilic and can directly scavenge ROS, lipid hydroperoxides, and protects the body against oxidative stress.

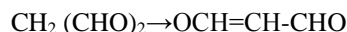
Malondialdehyde

Lipid peroxidation refers to the oxidative degradation of lipids. It is the process whereby free radicals remove electrons from the lipids in cell membrane, resulting in cell damage.

The process proceeds by a free radical chain reaction mechanism. It most often affects polyunsaturated fatty acids, because they contain multiple double bonds and methylene CH₂ groups that possess especially reactive hydrogen. As with any radical reaction, this reaction consists of three major steps; initiation, propagation and termination.

Of the many biological targets of oxidative stress, lipids are the most involved class of biomolecules. Lipid peroxidation gives rise to a number of secondary products. Malondialdehyde (MDA) is the principle and most studied product of polyunsaturated fatty acid peroxidation. This aldehyde is highly toxic molecule and a marker of oxidative stress.

Malondialdehyde mainly exists in the enol form:



The interaction of MDA with DNA and proteins has often been referred to as potentially mutagenic and carcinogenic. MDA reacts with deoxyadenosine and deoxyguanosine in DNA, forming DNA adducts.

MDA is an important marker used to study lipid peroxidation and assess the level of oxidative stress. Membrane lipids and lipids in circulating lipoproteins such as low density lipoprotein (LDL) can interact with reactive oxygen species resulting in lipid peroxidation which is known to cause damage to cell membrane.³

Vitamin C

Vitamin C or L-ascorbate is an essential nutrient for higher primates and a small number of other species. The presence of ascorbate is required for a range of essential metabolic reactions in all animals and plants.

The pharmacophore of vitamin C is the ascorbate ion. In the living organisms, ascorbate is an antioxidant, as it protects the body against oxidative stress and is also a cofactor in several vital enzymatic reactions.⁴

Vitamin C as an antioxidant

When ascorbate donates electrons, they are lost sequentially. The species formed after loss of one electron is a free radical, semi-hydroascorbic acid or ascorbyl radical. As compared to other radicals, ascorbyl radical is quite stable with a half-life of 10-5 seconds and is relatively unreactive; this is why ascorbate is a preferred antioxidant. In simple terms, a reactive and possibly harmful free radical can interact with ascorbate. The reactive free radical is reduced, and the ascorbyl radical formed in its place is less reactive. Reduction of a reactive with formation of a less reactive compound is sometimes called free radical scavenging or quenching. Ascorbate is therefore a good free radical scavenger due to its chemical properties. Ascorbyl radical with its unpaired electron is not a long lived species. Upon loss of a second electron the compound formed is dehydroascorbic acid. Vitamin C can be oxidized by many species that have potential to be involved in human diseases.⁵

METHODS

This study was conducted and ethically cleared at Lokmanya Tilak Municipal Medical College. The study involved 25 obese children for vitamin C, 20 obese children for Malondialdehyde (MDA) and 18 non obese children in the age group of 5-14 years, without any complications.

Inclusion criteria

- Both girls and boys in the age group of 5-14 years
- BMI range of 18.5-24.9 for non-obese children and >30 for obese children.

Exclusion criteria

- Patients with acute macrovascular complications
- Patients with PCOD and diabetes.

A self-completed questionnaire about obesity related characteristics was administered to all the children. The self completed questionnaire included questions about age, sex, height, weight, number of meals, mean number of hours per day spent on physical activity.

Vitamin C and Malondialdehyde (MDA), an important product of lipid peroxidation, were chosen as biomarkers to study oxidative stress in this study. Blood was collected in EDTA Vacutainers (4ml) for estimation of vitamin C, and in Plain Vacutainers (3ml) for estimation of MDA. Plasma obtained from the sample was separated for the estimation of ascorbic acid using 2,6-dichlorophenol indophenols dye by colorimetric method. Ascorbic acid is stable in plasma only for 30 minutes, so analysis should be started immediately after collection of samples.^{8,9} For the estimation of malondialdehyde, the

serum obtained from the sample was separated. It is done colorimetrically by MDA-TBA colored complex.¹⁰

RESULTS

Oxidative stress biomarkers were studied in obese and non-obese children in the age group of 5 to 14 years by assessing the levels of biochemical parameters such as Malondialdehyde and vitamin C (Ascorbic Acid) in Serum and plasma respectively. Children were categorized in obese and non-obese groups by body mass index (BMI). Children were categorized as obese when BMI is greater than or equal to 30 and are categorized as non-obese when BMI is in between 18.5-24.9. Twenty five obese children were selected for Vitamin C with the mean BMI of 30.63 and 18 obese children were selected for Malondialdehyde with the mean BMI of 31.05.

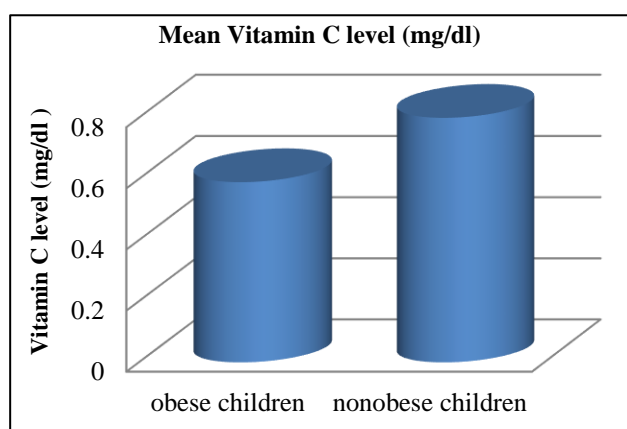


Figure 1: Mean vitamin C level in obese and non-obese children.

The results were subjected to statistical analysis. Unpaired “t-test” was applied and the statistical significance was established. Present study showed a significant lower level (p value <0.05) of vitamin C in plasma in obese children (Mean±SD level as 0.59±0.24) as compared to non-obese children (Mean±SD level as 0.80±0.08).

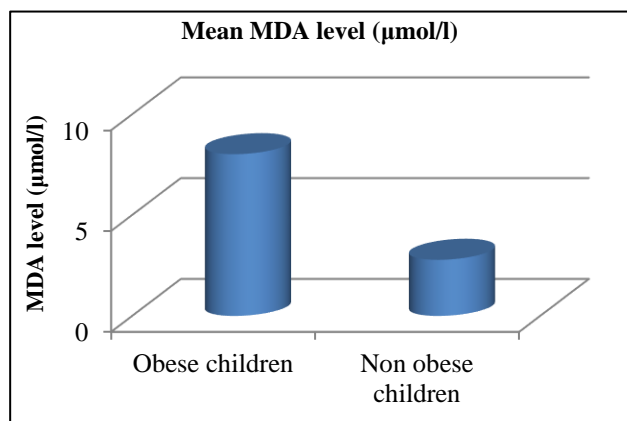


Figure 2: Mean MDA level in obese and non-obese children.

Present study showed a significantly higher values (p value <0.001) of serum malondialdehyde in obese children (Mean±SD level 8.02±1.80) as compared to non-obese children (Mean±SD level 2.78±0.85).

Table 1: Vitamin C and MDA levels in obese and non-obese samples.

Parameter	Obese children Mean±SD	Non-obese children Mean±SD	p value	Statistical significance
MDA	8.02±1.80	2.78±0.85	<0.001	Significant
Vitamin C	0.59±0.24	0.80±0.08	<0.05	Significant

Therefore, a significant lower level of antioxidant Vitamin C progresses the lipid peroxidation and thereby increasing the oxidative stress in children’s with obesity. Increased oxidative stress leads to oxidation of lipids and proteins, that can be cytotoxic and may cause plasmolemma leakage and dysfunction of membrane bound receptors and enzymes, signaling cascades and intercellular function. The most serious consequence of lipid or protein oxidation is DNA and nucleic acid damage and cell death.¹¹ Obesity is a major contributor to several other metabolic disturbances related to oxidative balance. Physical activity or exercise, dietary restrictions and surgical interventions reduce oxidative stress.

DISCUSSION

Present study showed a significant lower level of vitamin C in obese children (0.59±0.24) as compared to non-obese children (0.80±0.08). A similar trend was observed by Zhu YG et al, in their research.¹²

The lower levels of vitamin C increases oxidative stress in obese children and the possible explanation for that is vitamin C is a reducing agent or antioxidant due to its characteristics of donating an electron. Antioxidant neutralizes free radicals and protects tissue from damage. In body cells and fluids, vitamin C protects tissues from oxidative stress and thus plays an important role in preventing diseases. Vitamin C protects from oxidative stress, prevention of non-enzymatic glycosylation of proteins, and enhances arterial dilation through its effect on nitric oxide release. It also decreases lipid peroxidation, and inflammation. The anti-inflammatory property of vitamin C could be attributed to ability to modulate the NF-kB DNA binding activity and down-regulation in the hepatic mRNA expression for the interleukins and tumour factors.

Adequate tissue dietary, enzymatic and non enzymatic antioxidant defenses are critical to maintain antioxidant-proxidant balance in tissue. Perturbations to antioxidant defenses occur in obesity. Inadequacy of antioxidant defenses may begin with a low intake of protective antioxidants and phytochemicals in diet. Obese children

have a lower intake of phytochemical rich foods (fruits, vegetables, whole grains, legumes, wine, olive oil seeds and nuts) compared with non-obese children. Lower levels of antioxidants cannot scavenge excess ROS generated in obese children and thus there is increase in oxidative stress.¹³

Present study showed a significantly higher value of MDA in obese children (8.02±1.80) as compared to non-obese children (2.78±0.85). A similar trend is observed by Van Gaal et al, Codoñer-Franch P et al, Ustundag B et al, Lima et al, in their research.¹⁴⁻¹⁷

Obese children with higher levels of MDA are prone to oxidative stress because excess lipids react with ROS to produce lipid peroxides such as lipid hydroperoxides, which is hydrolyzed to a complex mixture of compounds that includes aldehyde as the predominant molecule. Excess MDA production has toxic effects on antioxidant enzymes in that MDA can modify amino acid side chains and oxidize thiol groups in antioxidant enzymes; these modifications often result in partial or complete loss of activity. Abnormal metabolism and metabolites in adipose tissue may generate and promote release of excessive amounts of proinflammatory and inflammatory cytokines and abnormal metabolism of other biochemical constituents could induce production and release of large amounts of O₂⁻, •OH, H₂O₂ and other ROS that increase oxidative stress and lipid peroxidation.

There are several other possible contributors to oxidative stress in obesity, including hyperglycemia, increased muscle activity to carry excessive weight, elevated tissue lipid levels, inadequate antioxidant defenses, chronic inflammation, endothelial ROS production, and hyperleptinemia. These factors are not mutually exclusive. Rather, obesity may involve some or all of these contributors to systemic oxidative stress. Depending on the status of the obese individual, one contributor may exert a greater oxidative stress effect than the others, but this contribution may change as the metabolic and physical status of the individual changes.² Regular regiment of increasing physical exercise to about 1-2 hours along with supplementation of antioxidant, lipoic acid could be the possible therapy to reduce oxidative stress.

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Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

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