ASSESSMENT OF ANTIOXIDANT STATUS IN PATIENTS WITH NON-ALCOHOLIC FATTY LIVER DISEASE

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INTRODUCTION

Non-Alcoholic Fatty Liver Disease (NAFLD) is an increasing global health concern, with an estimated prevalence of 20%-30% in Western countries and 15% in Asian countries. Oxidative stress is considered a major contributor as the “second hit” in the pathogenesis of NAFLD. Objectives: We aimed to compare serum total antioxidant, MDA and Nitric Oxide levels in patients with NAFLD and normal individuals. Methods and Materials: Patients with NAFLD (n=100) were compared to control subjects (n=100). Lipid peroxidation (Malondialdehyde) level was estimated using Thiobarbituric acid (TBA) method. Total antioxidant level was estimated using Phosphomolybdenum method. Nitric-oxide level was estimated using Griess-reagent method. Results are presented as mean + standard deviation value. Results: The mean serum level of total antioxidant in NAFLD individuals was found to be decreased when compared to normal individuals, whereas the levels of MDA and nitric oxide was found to be significantly increased compared normal individuals. Conclusion: This study may provide clinicians to draw different baselines for total antioxidant, Lipid peroxidation and Nitric oxide levels in patients with Non-Alcoholic Fatty Liver Diseases.

Keywords: Non-Alcoholic Fatty Liver Diseases, Oxidative stress, Malondialdehyde, Nitric oxide

Non-Alcoholic Fatty Liver Disease (NAFLD) is an increasing global health concern, with an estimated prevalence of 20%-30% in Western countries and 15% in Asian countries (Bellentani et al., 2010).

Non-Alcoholic fatty liver disease is an increasingly recognized condition that may progress to end-stage liver disease. The pathological picture resembles that of alcohol-induced liver injury, but it occurs in patients who do not abuse alcohol. A variety of terms have been used to describe this entity, including fatty-liver hepatitis, nonalcoholic Laennec’s disease, diabetes hepatitis, alcohol-like liver disease, and nonalcoholic steatohepatitis. Nonalcoholic fatty liver disease is becoming the preferred term, and it refers to a wide spectrum of liver damage, ranging from simple steatosis to steatohepatitis.
advanced fibrosis, and cirrhosis. Steatohepatitis (nonalcoholic steatohepatitis) represents only a stage within the spectrum of nonalcoholic fatty liver disease (Paul et al., 2002; Elmar Aigner et al., 2010; Guha et al., 2006; Michael Charlton, 2004).

Oxidative stress is known to play an important role in the onset of NAFLD. When pro-oxidant pathways generate more reactive species than can be consumed by antioxidant pathways (e.g., via protein disulfide isomerase or reduced glutathione peroxidase), oxidative stress occurs, with resulting accumulation of reactive oxygen species, chiefly superoxide and hydroxyl radicals plus hydrogen peroxide). Oxidative stress is considered a major contributor as the “second hit” in the pathogenesis of NAFLD and NASH, justifying the study of several antioxidants in NAFLD treatment (Shadid and Jensen, 2003).

Given the increased mortality rates in patients with NAFLD, the rising rates of obesity, diabetes, and metabolic syndrome in this population, finding an effective therapy is of utmost importance. In this study, our aim is to determine the oxidative stress markers in patients with NAFLD.

**MATERIALS AND METHODS**

**Groups**

**Control:** 100 healthy patients Serum was collected from K S Hegde Hospital, Mangalore.

**Study:** 100 Patients with Non-Alcoholic Fatty Liver Diseases serum was collected from K S Hegde Hospital, Mangalore.

**Inclusion Criteria:** Non fatty liver disease subjects who were diagnosed by USG.

**Exclusion Criteria:** Alcoholic fatty liver subjects.

- Patients suffering from hepatitis (ALT > 350).

**Blood Sampling:** 2 mL of blood collected in a plain bottle, centrifuged to separate serum.

**Oxidative Stress Markers:** Lipid peroxidation (Malondialdehyde) level was estimated using Thiobarbituric acid (TBA) method (Prieto et al., 1999). Total antioxidant level was estimated using Phosphomolybdenum method (Buege and Aust, 1978). Nitric-oxide level was estimated using Griess-reagent method (Wink et al., 1995).

**STATISTICAL ANALYSIS**

Results are presented as mean + standard deviation value. Student ‘t’ test was used to correlate between total antioxidant level and control groups. A ‘p’ value of 0.05 or less was considered significant.

**RESULTS**

The mean serum level of total antioxidant in NAFLD individuals was found to be decreased when compared to normal individuals, whereas the levels of MDA and nitric oxide was found to be significantly increased compared to normal individuals. Serum total antioxidant level in case of NAFLD was 146.05±24.89 to that of normal individuals 185.15±39.91. Showed in Table 1 and Figure 1.

The Serum Malondialdehyde level in case of NAFLD was 3.67±0.40 and that of control group 0.85±0.28. The Table 1 and Figure 2 shows the comparison values of MDA in case NAFLD and normal group.

The serum Nitric oxide level was increased in case of NAFLD patients 70.20±5.47 when compared to that of normal individuals 48.93±6.67. Values are shown in Table 1 and Figure 3.
DISCUSSION

The prevalence of NAFLD is continuously rising and represents a growing clinical problem. NAFLD is an increasingly recognized form of chronic liver condition affecting both children and adults within the wide spectrum of fatty liver diseases. Its incidence and prevalence are increasing, paralleling the increase in obesity and diabetes mellitus. It is well-known that lipid peroxidation and oxidative stress play significant roles in the pathogenesis of various diseases including chronic liver diseases. NAFLD is present in 10% to 24% of the general population in various countries (Elmar Aigner et al., 2010).

Increased oxidative stress is considered a key trigger in the pathogenesis of human NAFLD and one of the enzymes counteracting oxidative stress, copper/zinc (Cu/Zn) Superoxide Dismutase (SOD) depends on adequate copper availability, suggesting a potential link between copper availability and impaired antioxidant defence in NAFLD. (Elmar Aigner et al., 2012; Baquial and Sorenson, 1995).

Living organisms have evolved different molecules that speed up termination by catching free radicals and therefore protect the cell membrane. One important such antioxidant is vitamin E. Other anti-oxidants made within the body include the enzymes

Table 1: Comparison of Serum Total antioxidant, Nitric Oxide, MDA Levels in Normal and NAFLD

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal</th>
<th>NAFLD</th>
<th>'P' Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total antioxidant capacity (μg/mL)</td>
<td>185.15±39.91</td>
<td>146.05±24.89</td>
<td>&lt;0.0001(Significant)</td>
</tr>
<tr>
<td>Nitric Oxide (μM/L)</td>
<td>48.93±6.67</td>
<td>70.20±5.47</td>
<td>&lt;0.0001(Significant)</td>
</tr>
<tr>
<td>MDA (μM/L)</td>
<td>0.85±0.28</td>
<td>3.67±0.40</td>
<td>&lt;0.0001(Significant)</td>
</tr>
</tbody>
</table>

Note: *P<0.05 is statistically significant. Statistical comparison were performed by Student’s test. Data expressed as Mean±SD.
Superoxide dismutase, catalase and peroxidase. In addition, end products of lipid peroxidation may be mutagenic and carcinogenic. For instance, the end product malondialdehyde reacts with deoxyadenosine and deoxyguanosine in DNA, forming DNA adducts to them, primarily M1G (Fracasso et al., 2002).

Free radicals are electrically charged molecules, i.e., they have an unpaired electron, which causes them to seek out and capture electrons from other substances in order to neutralize themselves. Antioxidants are capable of stabilizing, or deactivating, free radicals before they attack cells. Antioxidants are absolutely critical for maintaining optimal cellular and systemic health and well-being. Hence body maintains complex system of enzymatic antioxidants such as catalase, SOD, peroxidases, etc., and non enzymatic antioxidants such as Vitamin C, E and glutathione, etc. Oxidative stress occurs as a result of increased oxidative metabolism. An inadequate intake of antioxidant nutrients may compromise antioxidant potential, thus compounding overall oxidative stress. Conditions associated with oxidative damage include heart disease, cancer, pulmonary disorders, ageing, etc. (Mataix et al., 1998; Srikrishna and Suresh, 2009).

An antioxidant is a molecule capable of inhibiting the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. They do this by being oxidized themselves, so antioxidants are often reducing agents such as thiols, ascorbic acid or polyphenols. Although oxidation reactions are crucial for life, they can also be damaging; hence, plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C, and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidase. Low levels of antioxidants, or inhibition of the antioxidant enzymes, cause oxidative stress and may damage or kill cells.

Lipid peroxidation refers to the oxidative degradation of lipids. It is the process whereby free radicals “steal” electrons from the lipids in cell membranes, resulting in cell damage. This process proceeds by a free radical chain reaction mechanism. It most often affects polyunsaturated fatty acids, because they contain multiple double bonds in between which lie methylene \(-CH_2-\) groups that possess especially reactive hydrogens. As with any radical reaction the reaction consists of three major steps: initiation, propagation and termination.

NO is an important signaling molecule in the body of mammals, including humans and is important intermediate in the chemical industry. (Hou et al., 1999) NO is an important messenger molecule involved in physiological and pathological processes within the mammalian body both beneficial and detrimental. Appropriate levels of NO production are important in protecting an organ such as the liver from ischemic damage. Chronic expression of NO is associated with various carcinomas and inflammatory conditions including juvenile diabetes, multiple sclerosis, arthritis and ulcerative colitis.
In tumor biology, nitric oxide has a complex array of concentration dependent action, including both inhibitory and promoting effect. It is thought that the levels of nitric oxide found in many human cancer lead to enhanced angiogenesis and tumor dissemination. A high salt intake was demonstrated to attenuate NO production, although bioavailability remains unregulated. (Osanai et al., 2002)

In the present study we observed that the serum level of total antioxidant in NAFLD individuals was found to be decreased when compared to normal individuals, whereas the levels of MDA and nitric oxide was found to be significantly increased compared normal individuals.

CONCLUSION
This study may provide clinicians to draw different baselines for total antioxidant, Lipid peroxidation and Nitric oxide levels in patients with Non-Alcoholic Fatty Liver Diseases.

REFERENCES


