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EVALUATION OF MALONDIALDEHYDE AND SUPEROXIDE DISMUTASE IN PATIENTS OF ALCOHOLIC LIVER DISEASE AND HEALTHY CONTROLS: A CROSS-SECTIONAL STUDY

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ABSTRACT

Background

Oxidative stress and an imbalance between oxidants and antioxidants are hallmarks of alcoholic liver disease (ALD), a major global cause of morbidity and mortality. Superoxide dismutase (SOD) and malondialdehyde (MDA) are important indicators for assessing the antioxidant and oxidative stress state in ALD. To comprehend the function of oxidative stress in ALD, this study intends to assess the blood levels of MDA and SOD in patients with ALD and compare them with healthy controls.

Methods

An analytical cross-sectional study was conducted involving 120 male participants. Serum MDA and SOD levels were measured using the Draper and Hadley method and the Marklund and Marklund method, respectively. Statistical analysis was done using an unpaired t-test with a significance level set at $p < 0.05$.

Results

The study included 120 male participants, divided into two groups: 60 patients with ALD and 60 healthy controls. All participants were age- and sex-matched. The serum MDA levels were notably higher in ALD patients (mean ± SD: 11.45 ± 3.01 nmol/ml) compared to controls (mean ± SD: 3.86 ± 0.73 nmol/ml) ($p < 0.001$). Similarly, serum SOD levels were notably elevated in ALD patients (mean ± SD: 13.69 ± 1.07 units/ml) compared to controls (mean ± SD: 5.04 ± 1.46 units/ml) ($p < 0.001$). A strong positive correlation was detected between MDA and SOD levels ($r_s = 0.988$, $p < 0.001$).

Conclusion

ALD patients exhibit enhanced oxidative stress as indicated by elevated serum MDA levels and a concomitant rise in antioxidant SOD levels. This suggests a compensatory mechanism against oxidative damage in ALD.

Recommendations

Future studies should focus on therapeutic interventions aimed at modulating oxidative stress and antioxidant defense mechanisms in ALD.

Keywords: Alcoholic Liver Disease, Oxidative Stress, Malondialdehyde, Superoxide Dismutase, Antioxidants, Lipid Peroxidation.
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INTRODUCTION

Alcoholic liver disease (ALD) is a major cause of morbidity and mortality that is becoming more common around the globe, especially in emerging nations such as India. In the West, alcohol intake continues to be the primary cause of cirrhosis. Drinking too much alcohol can cause several physical, mental, and psychological problems, making it a serious medical and societal issue. There is no artificial equivalent for the liver's functions, which are vital to survival [1]. Drinking alcohol excessively and continuously changes metabolic pathways and causes fat to accumulate in the liver, which produces reactive oxygen species (ROS) [2]. Since the liver is the major site of alcohol metabolism, it is especially susceptible to the harmful effects of alcohol metabolic byproducts, which can result in oxidative stress and liver injury through the creation of adducts and highly reactive molecules like ROS [3].

An imbalance between antioxidants and oxidants, known as oxidative stress, is linked to cellular damage and the development of ALD into liver fibrosis, which is characterized by cirrhosis, fatty liver, and alcoholic hepatitis. Lipid peroxidation is a crucial component of oxidative stress, which is caused by free radicals, the primary agents of oxidative damage in ALD. Free radicals destroy lipids, proteins, and DNA within cells [4]. This

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oxidative damage is indicated by lipid peroxidation products such as 4-hydroxynonenal (HNE) and malondialdehyde (MDA) [5]. Oxidative damage is fought by the body's defense mechanisms, also referred to as antioxidants. Among them are anti-superoxide radical enzymes such as superoxide dismutase (SOD) [6].

The quantity and duration of alcohol drinking increase progressively the risk of severe ALD [7]. Overconsumption of alcohol causes several illnesses, such as cancer, heart disease, and gastrointestinal disorders. ALD is becoming more common, especially in poorer nations [8]. Since there is presently no effective treatment for ALD other than abstinence from alcohol, preventive measures are necessary.

This study evaluated the serum levels of MDA and SOD in patients with ALD and compare them with healthy controls to identify the role of oxidative stress in ALD.

METHODOLOGY

Study design

An analytical cross-sectional study.

Study setting

The study was conducted on patients of Shimoga Institute of Medical Sciences, Shivamogga, Karnataka, India, spanning from December 2013 to May 2015 (18 Months).

Participants

Sixty age and sex-matched healthy controls and sixty cases of alcoholic liver disease were included in the study. Every patient gave their informed consent, and the college's.

Inclusion criteria

The study group consists of 60 male patients of ALD attending the medicine department on an IPD/OPD basis with a history of alcohol intake for more than 10 years, with a daily intake of about 80-100 grams/day.

Exclusion criteria

1. Severely ill patients with terminal-stage disease
2. Cases with multi-organ involvement
3. Suffering from hepatitis cirrhosis or jaundice not related to alcohol.
4. Patients suffering from diabetes mellitus and hypertension.

Bias

The study exhibits selection bias by including only male patients with long-term alcohol intake, limiting its generalizability. Confounding variables such as diet, smoking, and physical activity were not controlled, which can significantly impact oxidative stress and antioxidant levels. Additionally, measurement bias could arise due to variations in laboratory practices affecting the accuracy of MDA and SOD measurements.

Variables

The variable is alcohol consumption, defined as a history of over 10 years with a daily intake of 80-100 grams, serum levels of MDA and SOD, indicating oxidative stress and antioxidant status, respectively.

Sample collection method

About 8 ml of venous blood was drawn in fasting condition with aseptic precaution from a large peripheral vein (cubital vein) and collected in a plane vial. The serum was separated by centrifugation and kept at -39° c until analysis was carried out.

Serum was used for the estimation of MDA and SOD.

MDA was estimated by the Draper and Hadley method using thiobarbituric acid12. The absorbance is measured at 610 nm wavelength of light. Marklund and Marklund estimated serum SOD at an alkaline pH of 8.5 using the pyrogallol reagent. The auto-oxidation of pyrogallol is inhibited by superoxide dismutase (SOD), as evidenced by a rise in absorbance at 420 nm13.

Statistical analysis

The unpaired "t" test was used to assess the study results. Statistics were considered significant if the P value was less than 0.05.

Ethical considerations

The study protocol was approved by the Ethics Committee and written informed consent was received from all the participants.

RESULTS

Table 1. Serum MDA level (nmol/ml) in cases and controls.

	Cases (n=60)	**Controls (n=60)**
Mean ± SD	11.45 ± 3.01	3.86 ± 0.73
Median ± IQR	11.73 ± 5.14	3.78 ± 0.73
P value	<0.001	

Table no.1 depicts mean serum level and Median ± Interquartile range of MDA in controls and cases, P value is <0.001. The serum level of MDA is significantly elevated in cases compared to controls.

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Table 2. Serum SOD level (units/ml) in cases and controls

	Cases (n=60)	Controls (n=60)
Mean ± SD	13.69 ± 1.07	5.04 ± 1.46
Median ± IQR	13.86 ± 1.75	4.96 ± 2.15
P value	<0.001	

Table no. 2 show that mean serum level of SOD in controls and cases are 5.04± 1.46 and 13.69± 1.07 respectively. Median ± IQR in controls and cases are 4.96± 2.15 and 13.86± 1.75 respectively. P value < 0.001. So SOD level is significantly elevated in cases compared to controls.

Table 3. Correlation of MDA with SOD in cases.

	Spearman rank correlation coefficient (r_s)	P value
MDA with SOD	0.988	P<0.001

Table no. 3 shows that Spearman's rank correlation coefficient (rs) of MDA with SOD is 0.988. There is a strong positive correlation of MDA with SOD. As the serum level of MDA increases, the SOD level also increases.

DISCUSSION

In the study, serum MDA level, which is a marker of oxidative stress is significantly increased and there is also an increase in serum SOD level which acts as antioxidants in cases compared to controls. Many studies have shown similar results i.e. increase in serum MDA which acts as an oxidative stress marker as well as an increase in serum SOD which acts as an antioxidant. The generation of reactive oxygen species by alcohol metabolism causes lipid peroxidation leading to oxidative damage of polyunsaturated fatty acids(PUFA) which results in the production of reactive and toxic metabolites such as MDA. In the present study, the mean value of serum MDA in cases and controls is 11.45 nmol/ml and 3.86 respectively. The mean value of serum MDA level in ALD cases is increased when compared with controls. The increase is highly significant (p<0.001) and is by the other studies [9-12].

MDA resulting from lipid peroxidation of PUFA is used as an index of lipid peroxidation. MDA introduces cross-links in proteins which may induce profound alteration in their biochemical properties. Either exposure to more oxidative stress or an inefficient antioxidant defense of the cells leads to enhancement of the peroxidation reaction of cellular molecules. Fibrosis of the liver occurs mainly by activation of stellate cells, which is provoked by MDA. It has also been proposed that MDA could react physiologically with several nucleosides (deoxy-guanosine, deoxy-cytidine).

The amount of SOD is organ-specific and it is abundant in hepatic tissue. The enzyme superoxide dismutase catalyzes the breakdown of superoxide ions (O2•-) and provides the first line of defense against oxygen toxicity.

In the present study, the mean value of SOD in cases and controls are 13.69 1.07 and 5.04 1.46 respectively. The median interquartile range (IQR) of SOD in cases and controls are 13.86 1.75 and 4.96 2.15 respectively. The increase is highly significant (p< 0.001). The correlation coefficient (rs) of SOD with MDA is 0.988 and the p-value is <0.001. The correlation coefficient (rs) value indicates that serum SOD level is strongly correlated with MDA level. As the level of MDA increases, the SOD level also increases.

The present study is by the studies done by others in the past [13,14].

According to the current study, alcohol intake boosted SOD activity. Increased dismutation of superoxide to H2O2 is the outcome of overexpressing SOD. An increasing amount of data suggests that the onset of alcoholic liver disease may be linked to intermediates of oxygen decrease. In the liver, ethanol dramatically raises SOD activity. It might be brought on by elevated Mn-SOD gene expression or elevated Cu, and Zn-SOD activity in the liver.

Generalizability: The generalizability of the study findings is limited due to several factors. First, the study only included male participants with long-term alcohol consumption, excluding females and individuals with different drinking patterns or co-existing conditions like diabetes or hypertension. This restricts the applicability of the results to the broader population. Additionally, the study did not control for potential confounding variables such as diet, smoking, and physical activity, which can influence oxidative stress levels. The setting in a single medical institution in India further limits the external validity, as the findings may not be directly applicable to populations in other geographic regions or healthcare environments.

CONCLUSION

Alcoholic liver disease occurs because there is excessive and chronic consumption of alcohol and its toxic effects on the liver. In alcoholic liver disease patients, there is an increase in oxidative stress. The imbalance between pro-oxidants and anti-oxidants is the main reason for oxidative stress. Oxidative stress is brought on by the production of reactive oxygen species. The illness is progressive and a

leading cause of morbidity and death because of long-term, excessive alcohol use.

According to the current study, higher levels of antioxidants and oxidative stress-induced free radical production are the main causes of Alcoholic liver disease (ALD). Therefore, it is essential to keep antioxidant concentrations like SOD high to combat the oxidative stress that alcohol causes.

Limitations

The limitations of this study include a small sample population who were included in this study. Furthermore, the lack of a comparison group also poses a limitation for this study's findings.

Recommendation

Future studies should focus on therapeutic interventions aimed at modulating oxidative stress and antioxidant defense mechanisms in ALD.

Acknowledgment

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List of abbreviations

ALD: Alcoholic Liver Disease
SOD: Superoxide Dismutase
MDA: Malondialdehyde
ROS: Reactive Oxygen Species
PUFA: Polyunsaturated Fatty Acids
IPD: Inpatient Department
OPD: Outpatient Department
HNE: 4-Hydroxynonenal
SD: Standard Deviation
IQR: Interquartile Range
rs: Spearman's Rank Correlation Coefficient

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Conflict of interest

The authors have no conflicting interests to declare.

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