Original Article

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Biochemistry Section

Oxidative DNA Damage and Deranged Lipid Profile in Patients with Pulmonary Tuberculosis: A Cross-sectional Study

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ABSTRACT

Introduction: Tuberculosis (TB) is one of the major health problems and a leading cause of death from infectious diseases. Oxidative damage in tuberculosis results from biochemical interactions between Reactive Oxygen Species (ROS) and target biomolecules. Increased oxidative stress leads to elevated Oxidative Deoxyribonucleic Acid (DNA) damage in Pulmonary Tuberculosis (PTB) patients. It is important to study serum lipids and oxidative DNA damage as they affect the overall immune system of individuals.

Aim: To investigate deranged lipid profiles and oxidative DNA damage in PTB.

Materials and Methods: A single-centered, cross-sectional study was conducted at Grant Government Medical College, Mumbai from October 2016 to November 2017. A total of 50 PTB patients in the age group of 20-60 years and 50 healthy subjects were included in the study. Oxidative DNA damage was assessed using 8-hydroxy-2-deoxyguanosine (8-OHdG) as a biomarker with a highly sensitive Enzyme-linked Immunosorbent Assay (ELISA) kit, and lipid profiles were estimated. An Unpaired t-test was used to analyse the significance of the study parameters in the cases and control groups.

Results: The study involved 100 participants, predominantly males (63%), with most falling in the age group of 18-45 years (77%). The present study revealed significantly elevated levels (p-value <0.05) of 8-OHdG in patients; however, significantly reduced levels (p-value <0.05) of total cholesterol, Triglycerides (TG), High-density Lipoprotein cholesterol (HDL), Very Low-density Lipoprotein cholesterol (VLDL), and Low-density Lipoprotein cholesterol (LDL) were observed in cases compared to controls.

Conclusion: The present study observed an increase in the level of 8-hydroxy-2-deoxyguanosine (8-OHdG), indicating greater oxidative DNA damage in tuberculosis patients due to oxidative stress, leading to lipid profile derangement.

Keywords: Carcinogenesis, Deoxyribonucleic acid, Oxidative stress, Triglycerides

INTRODUCTION

Tuberculosis is the world's seventh leading cause of death [1]. India has the highest TB burden globally and accounts for nearly one-fifth (20%) of the global burden of TB, with two-thirds of cases in the South-East Asian Region. Every year, approximately 0.8 million people are newly found to be smear-positive TB cases, which are highly infectious. The annual risk of becoming infected with TB is 1.5%, and once infected, there is a 10% lifetime risk of developing TB disease [2,3].

According to the World Health Organisation (WHO), 10 million new TB cases and 1.5 million deaths were reported due to TB in 2018. Five countries, namely India, China, Indonesia, South Africa, and Nigeria, have the highest TB disease burden, with about one-fifth of global TB cases reported in India alone [4].

Active TB is an acute inflammatory condition associated with tissue injury due to the increased generation of Reactive Nitrogen Intermediates (RNI) and ROS as a consequence of phagocytic respiratory burst [5]. ROS can damage nucleic acids, lipids, proteins, and promote the progression of numerous cancers as well as coronary and carotid atherosclerosis. 8-hydroxy-2'-deoxyguanosine (8-OHdG) or 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) is one of the predominant forms of free radical-induced oxidative lesions and has therefore been widely used as a biomarker for oxidative stress and carcinogenesis [6-10].

Cholesterol is used to maintain the fluidity of cell membrane structure, participate in the activity of enzymes, and assist in the functions of phagocytosis and cell growth. The accumulation of cholesterol in blood vessels narrows the blood vessels and causes atherosclerosis [11-13].

The inability of macrophages to uptake *Mycobacterium tuberculosis* (MTB) due to a low cholesterol content in their cell membrane might constitute a key defect in the host defense system against TB [14]. The level and function of the lipid profile are negatively affected by pulmonary TB [15].

There have been very few studies done to assess deranged lipid profiles and oxidative DNA damage, so conducting the present research can help determine their role in the prognosis of tuberculosis patients [16,17].

Hence, the present study aimed to investigate deranged lipid profiles and oxidative DNA damage in PTB.

MATERIALS AND METHODS

A single-centered, cross-sectional study was conducted at the Grant Government Medical College, Mumbai from October 2016 to November 2017. The study was approved by the Institutional Ethics Committee (IEC/106/2018), and written informed consent was obtained from all patients included in the study.

Inclusion criteria: A total of 50 newly diagnosed (sputum positive) patients of PTB, in the age group of 20 to 60 years, were included. A group of 50 age and sex-matched healthy controls with no complaints and no known diseases were also included.

Exclusion criteria: Patients with Multidrug-resistant (MDR) TB, patients with extrapulmonary TB, and patients with TB and Human Immunodeficiency Virus (HIV) infection were excluded.

Sample size calculation: For sample size calculation, the Power analysis method was used, and results were obtained from OpenEpi, Version 3, an open-source calculator-SS Mean, maintaining a 95% confidence interval. The sample size was calculated based on a previously published study by Wagh V et al., [18].

Study Procedure

For data collection, 8 mL of venous blood samples were collected from the antecubital vein of the subjects under all aseptic precautions. All samples were centrifuged at 4500 rpm for five minutes to obtain clear serum. Parameters estimated and the methods used has been provided in [Table/Fig-1] [19-21].

Parameters estimated	Name of the method	Cut-off range
Serum total cholesterol	Cholesterol oxidase peroxidase method	<200 mg/dL
Serum triglyceride	Glycerophosphate-oxidase peroxidase method	<150 mg/dL
Serum HDL cholesterol	Direct method	<40 mg/dL
LDL cholesterol and VLDL cholesterol	Friedewald's equation	<130 mg/dL

[Table/Fig-1]: Parameters estimated [19-21].

Oxidative DNA damage was studied using Stress Marq's 8-OHdG ELISA kit, which is a competitive assay used for the quantification of 8-OHdG in urine, cell culture, plasma, and other sample matrices. The ELISA utilises an 8-hydroxy-2-deoxyguanosine-coated plate and an Horseradish Peroxidase (HRP)-conjugated antibody for detection, allowing for an assay range of 0.94-60 ng/mL, with a sensitivity of 0.59 ng/mL. Other highlights of this kit include a quick incubation time of 60 minutes, stable reagents, and an easy-to-use protocol [19].

STATISTICAL ANALYSIS

Descriptive statistical analysis was carried out in the present study. Results on continuous measurements were presented as Mean±Standard Deviation (SD). An Unpaired t-test was used to study the significance of study parameters in cases and control groups. A p-value of <0.05 was considered statistically significant. For statistical analysis, the "GraphPad QuickCal Version 7" software was used. Microsoft word and excel were used to generate tables and graphs.

RESULTS

The study was conducted on 100 participants. The majority were males (63%), and most of them belonged to the age group 18-45 years (77%). No significant difference was found in age and gender distribution among cases and controls [Table/Fig-2].

Variables	n (%)	Cases	Controls	p-value
Gender				
Males	63 (63%)	30	33	0.35
Females	37 (37%)	20	17	
Age distribution (in years)				
18-45	77 (77%)	49	28	0.28
>45	23 (23%)	1	22	

[Table/Fig-2]: Age and gender-wise distribution of study participants.

A significant decrease in total cholesterol (p-value=0.02), Triglycerides (TG) (p-value=0.007), HDL (p-value=0.0001), VLDL (p-value 0.009), and LDL levels (p-value 0.009) in cases compared to control is shown in [Table/Fig-3].

Lipid parameters	Cases Mean±SD (mg/dL)	Controls Mean±SD (mg/dL)	p-value (Unpaired t-test)
Total cholesterol	166.26±28.15	176.9±18.34	0.02
TG	85.68±30.94	99.58±17.85	0.007
HDL	37.54±13.57	59.66±12.89	0.0001
VLDL	17.1±6.63	20±4	0.009
LDL	101.34±19.59	113±24	0.009

[Table/Fig-3]: Lipid parameters in cases and control.

There was a significant increase (p-value=0.0001) in the levels of 8-hydroxy-2-deoxyguanosine in cases (5.8±4.05 ng/mL) compared to controls (0.8±0.21 ng/mL) [Table/Fig-4].

Parameter	Controls Mean±SD (ng/mL)	Cases Mean±SD (ng/mL)	p-value (Unpaired t-test)	Difference
8-OH-dG	0.8±0.21	5.8±4.05	0.0001	Significant

[Table/Fig-4]: Analysing and comparing 8-OH-dG among case and control.

DISCUSSION

In the present study, there was a significant (p-value <0.05) decrease in total cholesterol, TG, HDL, VLDL, and LDL levels in cases compared to controls. These findings are similar to those of Sushilendu V et al., where it was found that all lipid parameters were significantly low in cases infected with pulmonary TB [20]. Taparia P et al., studied that all lipid parameters were significantly (p-value <0.05) low in both newly diagnosed and relapse cases of PTB compared to controls [21].

Metwally M and Raheem HA, found that in pulmonary TB, both cholesterol and TG were significantly lower before treatment than in controls (p-value <0.05 and p-value <0.01, respectively) [22]. This indicates that hypocholesterolemia can be seen as a consequence of TB. Wagh V et al., Akpovi DC et al., and Oyedeji SO et al., studied that *Mycobacterium tuberculosis* (MTB) activates invaded macrophages resulting in a free radical burst. High serum levels of free radicals and a high concentration of lipid peroxidation products are characteristics of patients with advanced tuberculosis. The peroxidation could cause a reduced concentration of serum lipids similar to what is seen in the present study [18,23,24].

In the present study, there was a statistically significant increase in the levels of 8-hydroxy-2-deoxyguanosine in cases compared to controls (p-value <0.001). The 8-hydroxy-2-deoxyguanosine level was higher in cases, with a mean±SD of 5.8±4.05 ng/mL, while in controls, the mean±SD was 0.8±0.21 ng/mL. The increased level of 8-OH-dG suggests more oxidative damage in patients with tuberculosis.

Pilger A and Rüdiger HW identified that interactions between ROS and biomolecules can cause oxidative DNA damage. ROS can damage nucleic acids, lipids, and proteins; this damage plays a significant role in the etiology and progression of numerous cancers, as well as coronary and carotid atherosclerosis. Although many damaged DNA lesions have been identified, authors have chosen 8-OHdG as the biomarker of oxidative DNA damage [25].

In the present study, there was statistically significant increase in levels of 8-hydroxy-2-deoxy guanosine in cases as compared to controls (p<0.001). The 8-OHdG level is higher in cases which was 5.8±4.05 ng/mL whereas in controls it was 0.8±0.21 ng/mL, increased level of 8-OH-dG suggest more oxidative damage in patients with tuberculosis. Kolgiri V et al., suggested 8-OHdG levels acts as an oxidative stress marker as these levels are increased in HIV patients as compared to controls result in somatic mutation and is the driving force behind carcinogenesis [26].

Rothschild BM et al., Kryston TB et al., and Cooke MS et al., studied that the balance between oxidants and antioxidants shifts in favour of the former. When a condition of oxidative stress arises, widespread modification of molecules such as lipids and proteins occurs [27-29]. Nucleic acids and their precursors (deoxy) ribonucleotide pools are particular targets. More than 70 damage products have been described, whose presence can have significant implications for cell function. For example, in addition to producing mutations, oxidatively modified DNA can lead to alterations in cell signalling and gene expression, promote microsatellite instability, and accelerate telomere shortening. As a result, oxidative stress has been implicated in a wide variety of pathological conditions, including cancer, cardiovascular disease, aging, and neurodegenerative diseases. The most widely studied biomarker of DNA oxidation is 8-OHdG [30-31].

Cooke MS and Evans MD, reported that in *Mycobacterium tuberculosis* infection, ROS are generated by host phagocytic cells as part of the host defense system. Mycobacterium, being a successful intracellular pathogen, has evolved a number of antioxidant strategies to avoid being killed by the ROS produced by host cells. However, an excess of these ROS can lead to necrosis of granulomatous lesions, causing the dissemination of bacilli from the granulomas [30].

Loft S and Poulsen HE, found that understanding this complex interplay between oxidative stress generated during tuberculosis and the antioxidant mechanisms employed by the host and Mycobacteria is crucial for developing new preventive and therapeutic strategies for tuberculosis [31].

There is a potential supplemental role for micronutrients and other antioxidants in the management of both drug-sensitive and drug-resistant tuberculosis. Antioxidants may also have a beneficial role in the prevention and treatment of drug toxicity, particularly hepatotoxicity due to antitubercular drugs.

Limitation(s)

The limitations of the present study are that the socio-economic status was not taken into consideration. Additionally, Body Mass Index (BMI) was not assessed in the present study.

CONCLUSION(S)

Mycobacterium tuberculosis causes PTB, a chronic inflammatory lung illness. Lipids and their metabolites exert a beneficial influence on TB resistance through the immune system. In the current study, it was found that tuberculosis induces oxidative DNA damage, which can be measured using 8-OHdG as a biomarker. A decrease in the lipid profile indicates that cholesterol is necessary for proper immune system function, as immunity is reduced in tuberculous infections.

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