

ROLE OF BILIRUBIN AS A POTENTIAL ANTIOXIDANT IN SMOKERS

Biochemistry

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ABSTRACT

Introduction: Globally, smoking causes about 71% of lung cancer, 42% of chronic respiratory diseases and about 10% of cardiovascular disease. Smokers are exposed to potential oxidative damage. This leads to depletion of the body's defense against oxidative stress, like decrease in the levels of Paraoxanase-1. This study aimed to assess the antioxidant property of Bilirubin in comparison to Paraoxanase-1 in smokers.

Materials & methods: This case control study was carried out at Govt. Kilpauk Medical College Hospital after obtaining ethical committee approval and informed consent. This study involves 2 groups with 90 chronic smokers and 90 apparently healthy individuals who are not smokers. Serum Bilirubin and Paraoxanase-1 levels were estimated and compared between these two groups. Correlation between Bilirubin and Paraoxanase-1 was analyzed. Statistical analysis was done using SPSS package version 20.

Results: The mean value for Bilirubin is 0.566 $\pm$ 0.03 mg/dL in smokers and 0.620 $\pm$ 0.05 mg/dL in non-smokers. There is significant difference in bilirubin levels between smokers and non smokers (p value - 0.049). The mean value for Paraoxanase-1 is 3.291 $\pm$ 0.75 ng/dL in smokers and 4.513 $\pm$ 0.96 ng/dL in non-smokers. There is significant difference in Paraoxanase-1 levels between smokers and non smokers (p value - 0.025). The scatter plot between Paraoxanase-1 and Bilirubin shows no significant correlation.

Conclusion: The Paraoxanase-1 and Bilirubin values in smokers are considerably reduced in comparison to that of non-smokers. This shows that Paraoxanase-1 and Bilirubin exhibits potential antioxidant properties. Bilirubin estimation is inexpensive and can be easily measured rather than measuring other known antioxidants such as PON-1. Role of Bilirubin as a marker to detect the oxidative stress levels in smokers is established in this study.

KEYWORDS

Bilirubin, Paraoxanase -1, Antioxidant, Smokers

INTRODUCTION

About 11.2% adults in India are chain smokers^{[1][2]}. In a recent survey conducted in the city of Chennai, about 21% adults smoke tobacco.^[3] Increased and widespread use of tobacco has made it the second major cause of death in the world.^{[4][5]}

Each year tobacco smoking kills about 5 million people worldwide. Smoking is a major risk factor for cardiovascular disease, lung cancer, mouth cancer, COPD and various other pulmonary diseases. Smoking also exacerbates various conditions that affects lungs such as Covid - 19 and impedes the successful treatment of the same.

Chronic cigarette smoking is related to increased free radical production and antioxidant depletion, in humans. Reactive elements in cigarette smoke have been shown to induce platelet activation, lipid/lipoprotein oxidation and DNA adduct formation.^[6]

Substances that neutralise the degenerative effect of free radical-induced lipid peroxidation are generally grouped as antioxidants and are responsible for cellular protection against oxidative stress. The antioxidative capacity of serum is related to antioxidant property of the enzymes Superoxide Dismutase, Glutathione Peroxidase and paraoxanase-1 (PON-1).

Human serum paraoxanase-1 (PON-1) is a polymorphic enzyme that plays an important role in lipid metabolism, thereby in cardiovascular disease and atherosclerosis. In vitro, PON1 significantly decreases lipid peroxide generation during LDL oxidation and preserves HDL modification by lipid peroxide. Studies have shown that smoking is related to low PON-1 activity.^[7]

Bilirubin, an end product in the normal catabolic pathway of Heme has been shown to have antioxidant activity as it suppress lipid oxidation, especially of LDL cholesterol.

A bilirubin-biliverdin cycling mechanism has been proposed to explain the possible antioxidant property, to show how bilirubin reacts with oxyradicals generating biliverdin, which is then reduced back to bilirubin by NADPH/biliverdin-reductase activity.^[8]

Bilirubin was found to be a free radical scavenger by donating a

hydrogen atom attached to the C-10 bridge of the tetrapyrrole molecule to form a carbon-centred radical and an inhibitor of superoxide production by mechanism employing the inhibition of NADPH oxidase^[8]

This study aimed at establishing role of Bilirubin as a marker to detect the oxidative stress levels in smokers similar to that of Paraoxanase-1.

MATERIALS AND METHODS

This Case-control study was conducted in the Department of Biochemistry in association with Department of General Medicine, Government Kilpauk Medical College Hospital, Chennai. The study protocol and all procedures concerning human subjects were approved by the Institutional Ethical Committee. Informed written consent was obtained from the study population.

The case group consisted of 90 Chain smokers (4 pack years). The control group consisted of 90, healthy individuals who are non-smokers. With exclusion criteria being patients with Diabetes mellitus, hypertension, chronic obstructive pulmonary disease, liver or renal disease, malignancy, haematological disorders and individuals having a first degree relative diagnosed with coronary artery disease.

For the study, 5 ml of 10 hours Fasting Venous Blood sample was collected under sterile aseptic conditions from the ante cubital vein in red topped vacutainer tubes. Serum was separated after centrifugation at 3000 rpm for 10 minutes and aliquoted into 3 eppendorfs. Stored at -20°C and were not thawed until the batch was analyzed for Serum Bilirubin and PON-1.

Estimation of serum total bilirubin levels was done using diazo method in automated COBAS C311 clinical chemistry analyser. COBAS calibrator and precicontrol level 1 & 2 were used for checking internal quality. The Coefficient of Variation of all the analytes performed was within the prescribed limits in accordance with CLSI. Serum Paraoxanase - 1 level was estimated Using ELISA method.

Statistical analysis

Statistical analysis was performed using SPSS package version 20. Spearman's rank correlation is used for univariate analysis. The groups are compared using paired sample T test. P<0.05 is considered to be

statistically significant.

RESULTS

The smokers and non smokers in the study population were matched for their age and evaluated statistically

Table 1: Paraoxonase - 1 (PON-1) and bilirubin values in smokers.

	PON-1 SMOKERS	BILIRUBIN-SMOKERS
Mean:	3.29	0.56
Median:	2.40	0.50
Std. Dev:	0.75	0.03
Minimum Value:	0.09	0.50
Maximum Value:	11.25	1.00

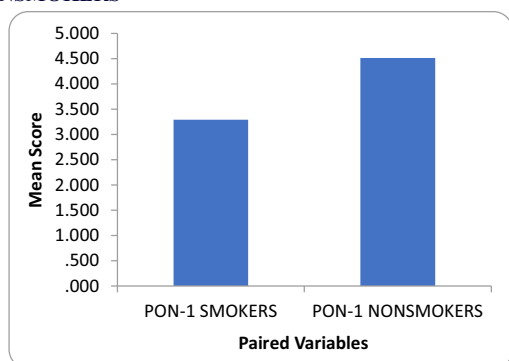
Table 2: Paraoxonase - 1 (PON-1) and Bilirubin values in non-smokers

	PON-1 NONSMOKERS	BILIRUBIN-NONSMOKERS
Mean:	4.51	0.62
Median:	3.28	0.50
Std. Dev:	0.96	0.05
Minimum Value:	0.53	0.50
Maximum Value:	23.54	0.50

DISCUSSION

The mean value for Paraoxonase-1 in smokers is 3.291 ng/dL and the mean value in non-smokers is 4.513 ng/dL. The p value is 0.025 and the difference is statistically significant (Figure 1).

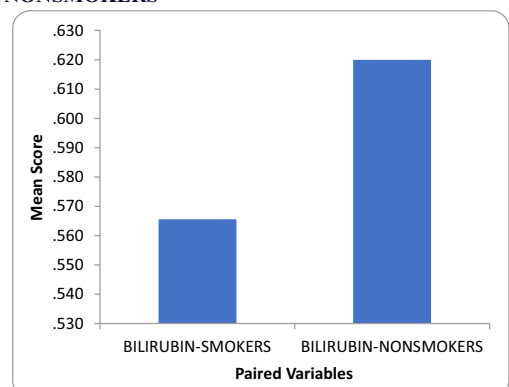
Figure 1: Paired Samples T-Test of PON-1 in SMOKERS and NONSMOKERS



The observed difference is statistically significant with p value as 0.025.

The level of PON-1 is reduced in smokers to that of non-smokers which implies that PON-1 plays an antioxidant role and prevents peroxidative damage to cell membranes. This is in accordance to the study conducted by Nishio et al. which demonstrated the inhibition of Paraoxonase-1 enzyme activity by smoke extracts in healthy subjects. He also showed that inhibition was time and dose dependent.^{[6][9][10]} An analysis of bilirubin values showed a mean of 0.566 mg/dL and 0.620 mg/dL in smokers and non-smokers respectively. The p value is 0.049 and the difference is statistically significant (Figure 2).

Figure 2: Paired Samples T-Test of BILIRUBIN in SMOKERS and NONSMOKERS



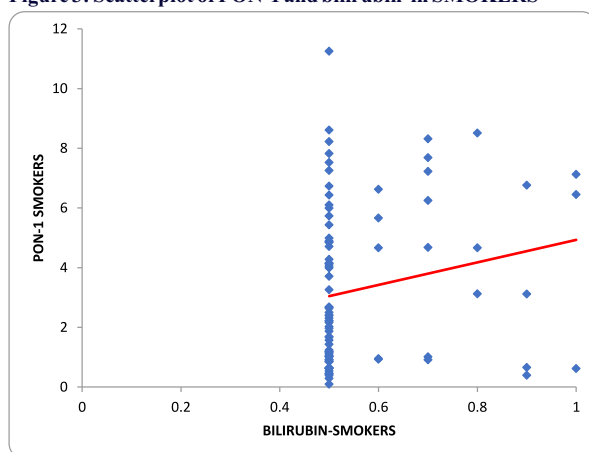
The observed difference is statistically significant with p value as 0.049.

This is in accordance with Jaeseong Jo et al's study on Korean men that cigarette smoking decreases total serum bilirubin.^[11]

In another study, elevated bilirubin levels provided a better index of disease protection than high-density lipoprotein cholesterol^[12] In a meta-analysis of 11 studies, the risk of atherosclerosis was diminished in individuals with elevated bilirubin levels. The risk of carotid plaques was reduced by about a third in individuals with elevated bilirubin levels^[13]. The HO/BVR (Heme oxygenase/Biliverdin Reductase) pathway has important roles other than its antioxidant activities. Carbon monoxide, a product of the HO reaction, can act as a neurotransmitter and anti inflammatory/anti proliferative molecule^{[11][14]}. Bilirubin can inhibit oxidant associated-neutrophil chemo taxis^{[15][16][17]}

The scatter plot between PON-1 and Bilirubin shows a p value of 0.071 with no statistically significant correlation (Figure 3)

Figure 3: Scatterplot of PON-1 and bilirubin in SMOKERS



There is no observed correlation

CONCLUSION

The PON-1 value in smokers is considerably reduced in comparison to that of non-smokers. This shows that PON-1 is an effective antioxidant. The bilirubin value in smokers is also considerably reduced in comparison to that of non-smokers. This shows that like paraoxonase-1, bilirubin also exhibits potential antioxidant property. Therefore bilirubin can be used as a marker to assess the complications that may occur in smokers due to increased oxidative stress. Since bilirubin is inexpensive, it can be easily measured in laboratories rather than measuring other known antioxidants such as PON-1.

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