

Comparing the Levels of Acute-Phase Reactants Between Smoker and Nonsmoker Diabetic Patients: More Predicted Risk for Cardiovascular Diseases in Smoker Compared to Nonsmoker Diabetics

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Abstract- Due to a close link between cardiovascular disorders and increased acute phase responses, it is now proposed the relation of total sialic acid (TSA) and C Reactive Protein (CRP) as main components of acute phase proteins and cardiovascular risk profiles such as diabetes mellitus and smoking. We hypothesized that the elevation in the level of TSA along with other prototype acute phase reactants such as CRP is expected more in the coexistence of diabetes and smoking than in diabetes mellitus alone. Ninety diabetic patients were randomly selected and entered into this case-control study. Using block randomization method, the patients were randomly assigned into smokers (n=45) and nonsmokers (n=45). A group of ten healthy individuals was also included as the control. The serum levels of TSA, CRP, iron, and hemoglobin were measured by the specific techniques. Comparing laboratory parameters across the three groups indicated significantly higher levels of TSA and CRP in smoker diabetics as compared to non-smoker diabetics and the healthy controls, while there was no difference in other parameters including serum iron and hemoglobin. A significant positive correlation was also revealed between TCA and CRP ($r=0.324$, $P=0.030$), but no significant association was found between other parameters. In the background of smoking, increasing the level of both TSA and CRP is predicted more than the existence of diabetes mellitus alone. In fact, the increase in these biomarkers is more predictable in smoker than in nonsmoker diabetics. This finding emphasizes the increased risk for cardiovascular disorders in smoker compared to non-smoker diabetics.

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Introduction

There is now a growing body of evidence on a significant link between cigarette smoking and increased risk of type II diabetes mellitus (1-4). About 12% of all diabetics are notably attributable to cigarette smoking (5). It has been also estimated the increased likelihood of occurring diabetes mellitus in 45% of male smokers and 74% of female smokers (6). More interestingly, some definitive evidence are available at increased risk for diabetes following exposure to secondhand smoking (7-10). The pathophysiological fundamentals of this link have been widely under investigating. It has been recently shown a close association of smoking with the increase of insulin resistance (11) as well as with

deterioration of glucose metabolism by the destruction of glucose regulatory pathways in the pancreas (12,13). The molecular effects of smoking on glucose metabolism have been also studied. It has been clearly revealed that cigarette smoking can activate inflammatory pathways and stimulate oxidative stress leading alteration of enzymes involving glucose and lipids metabolism (14,15). In total, due to synergistic effects of diabetes and smoking of heart and lungs, it can be predicted high risk for cardiovascular disorders among diabetic smokers.

Sialic acid is terminal part of carbohydrate chains of glycolipids and glycoproteins constituting the main component of several regulatory enzymes and hormones (16). About half of total sialic acids (TSA) in serum

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originate from the acute-phase proteins, and thus elevation of this marker is an important sign of increasing the levels of acute-phase proteins (17). Due to the close link between cardiovascular disorders and increased acute phase responses, it is now proposed the relation between TSA as the main component of acute phase proteins and cardiovascular risk profiles such as diabetes mellitus, hyperlipidemia and even smoking-related defects (18). In the present study, we hypothesized that the elevation in the level of TSA along with other prototype acute phase reactants such as C-Reactive Proteins (CRP) is expected more in the coexistence of diabetes and smoking than in diabetes mellitus alone. In this regard, we compared the levels of TSA, CRP, serum iron and hemoglobin between smoker and nonsmoker diabetics.

Materials and Methods

Study population

The study endpoint was to assess the impact of cigarette smoking on serum levels of TSA, CRP, iron, and hemoglobin in type II diabetic patients. To achieve this goal, 90 diabetic patients (with hemoglobin A1c >7%) were randomly selected and entered into this case-control study. Using block randomization method, the patients were randomly assigned into smokers (n=45) and nonsmokers (n=45). Those with the history of cancers, liver diseases, anemia, renal defects, or any pieces of evidence of acute or chronic inflammatory disorders were all excluded. A group of ten healthy individuals without history of diabetes or any pieces of evidence of metabolic disorders was also included as the control. This study was undertaken after approval by the institutional ethical committee overseeing human studies. Experiments were done in accordance with Helsinki Declaration of 1975.

Study measurement

Baseline characteristics and clinical information were collected by interviewing the patients and controls. Venous blood samples were taken from all subjects immediately after enrollment in tubes containing EDTA and transferred to a single laboratory for assessing study parameters. Blood samples were centrifuged at 2000×g for 10 min. The technical method of assessing serum level of TSA was described in detail previously (19). Serum TSA was measured by a spectrophotometric assay. Briefly, serum 400 µl was mixed with 3.0 ml of

5% TSA for 5 minutes at 100° C and the tubes were covered with marbles. The tubes were cooled and centrifuged for 4 minutes at 1400 RPM. The supernatant (2.0 ml) was mixed with 400 µl each of acid reagent and diphenylamine reagent. The contents were mixed using a vortex mixer, covered with marbles and placed in a boiling water bath for 30 minutes. Development of a purple color was measured on a spectrophotometer at 525 nm. Finally, the level of TSA was measured after comparing with standard curve. Hemoglobin level was measured using an automated cell counter ((Pishtaz-e-Teb Co, Iran). Serum level of the iron level was measured by atomic absorption spectrometry (Pishtaz-e-Teb Co, Iran). The level of CRP has also measured immunoassay method (Pishtaz-e-Teb Co, Iran).

Statistical analysis

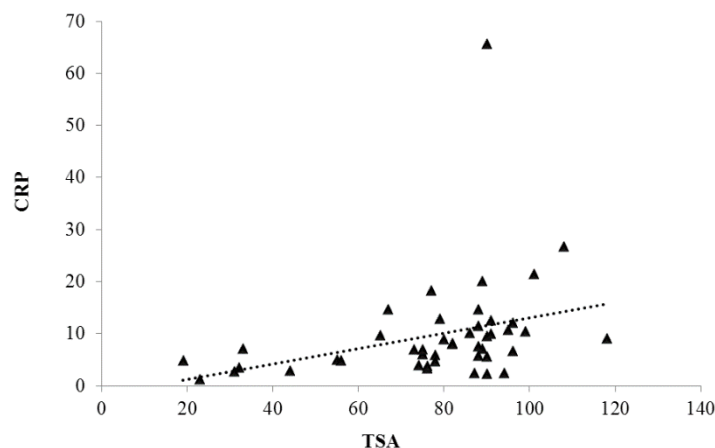
Results were presented as mean±standard deviation (SD) for quantitative variables and were summarized by absolute frequencies and percentages for categorical variables. Normality of data was analyzed using the Kolmogorov-Smirnoff test. Categorical variables were compared using chi-square test or Fisher's exact test when more than 20% of cells with expected count of less than 5 were observed. Quantitative variables were also compared with ANOVA test or Kruskal-Wallis H test. The correlation between the study parameters was assessed using the Pearson's correlation test. For the statistical analysis, the statistical software SPSS version 16.0 for windows (SPSS Inc., Chicago, IL) was used. P values of 0.05 or less were considered statistically significant.

Results

Due to the results of Kolmogorov-Smirnoff test, all study parameters were considered to have normal distribution, and thus parametric tests were employed for the analysis. As shown in Table 1, comparing laboratory parameters across the three groups indicated significantly higher levels of TSA and CRP in smoker diabetics as compared to non-smoker diabetics and the healthy controls, while there was no difference in other parameters including serum iron and hemoglobin across the three groups. Regarding the correlation between the laboratory indices, a significant positive correlation was revealed between TCA and CRP ($r=0.324$, $P=0.030$) (Figure 1). However, no significant association was found between other parameters (Table 2).

Table 1. Comparing serum TCA, CRP, iron and hemoglobin levels across the study groups

Groups	Diabetic smokers (n=45)	Diabetic nonsmokers (n=45)	Healthy controls (n=10)	P
C-Reactive protein	9.67±1.50	4.67±0.36	1.46±0.16	0.003
Iron	108.29±4.84	110.00±3.84	115.00±2.49	0.772
Hemoglobin	15.83±0.18	16.10±0.14	15.04±0.23	0.096
Sialic acid	77.38±3.31	35.82±1.78	29.50±3.45	< 0.001

**Figure 1.** Correlation between TCA and CRP**Table 2. Correlation between different laboratory parameters**

Groups	C-Reactive protein (r, P)	Iron (r, P)	Hemoglobin (r, P)	Sialic acid (r, P)
C-Reactive protein	--	(-0.180, 0.237)	(0.016, 0.918)	(0.324, 0.030)
Iron	(-0.180, 0.237)	--	(0.194, 0.201)	(-0.152, 0.320)
Hemoglobin	(0.016, 0.918)	(0.194, 0.201)	--	(-0.071, 0.643)
Sialic acid	(0.324, 0.030)	(-0.152, 0.320)	(-0.071, 0.643)	--

Discussion

Our study could clearly found higher concentrations of both TSA and CRP biomarkers in smoker diabetics when compared to nonsmokers and healthy individuals. In other words, smoking could be a determinant of increasing these markers in diabetics than in healthy subjects. Because of the existence of this effect, the synergistic effects of both smoking and diabetes through increasing TSA and CRP on acute reactants responses can be expected. Another important finding in our observation is a direct association between TSA and CRP indicating a common ring between the metabolic pathways of these two markers.

Similar to our survey, some other studies could demonstrate a close link between smoking and TSA level. As shown by Crook *et al.*, (20) plasma TSA was also significantly related to the presence of smoking even after adjusting for diabetes duration and control. In

their study, the level of smoke habit was directly associated with the level of TSA so that the probability of increasing the level of this marker was expected more in current smokers than in Ex-smokers and nonsmokers. In another study by Chen *et al.*, (21), smoking was significantly associated with the level of TSA in a multivariable regression model including other correlates of diabetes and cardiovascular disorders. Some studies could determine main factors that affect the level of TSA in plasma. As shown by Ponnio *et al.*, (22), the level of TSA was not affected by increasing age in men but could be potentially affected by female menopause. Also, the presence of hypertension was closely associated with increase the level of TSA. In their study, also, TSA level and smoking showed a significant correlation in male, but not in females. More interestingly, the association between smoking and TSA level directly depended on the number of cigarettes per day. However, the gender-difference in this association

remained unknown.

Unlike to the hypothesized association between smoking and level of TSA, the association between CRP level and smoking has been clearly demonstrated. Several studies showed association between CRP level and two smoking and diabetes mellitus states even independent to each other (23-27). These evidence emphasize and reinforce the pro-inflammatory impact of smoking particularly in the background of diabetes mellitus (28). Even, it has been shown higher level of CRP in current smokers than in ex-smokers (29,30). Along with the definitive effect of positive CRP as an independent risk factor for diabetes mellitus (31), the doubled effect of the coexistence of smoking and diabetes on CRP level can be also explainable.

It can be finally concluded that coexisting diabetes and smoking can amplify increasing the level of both TSA and CRP. In fact, it seems that the increased level of both biomarkers can effectively predict the occurrence of cardiovascular disorders through their association with two major risk profiles including diabetes and smoking. As an important finding, in the background of smoking, increase the level of both TSA and CRP is predicted more than the existence of diabetes mellitus alone.

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