

BLOOD CHOLESTEROL AND LEPTIN LEVELS IN MALE SMOKING AND NON-SMOKING PATIENTS WITH DIABETES MELLITUS

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ABSTRACT

In view of the controversial reports on the precise role of blood cholesterol and leptin levels in diabetes mellitus (DM) and smoking, it was planned in the present study to determine blood levels of cholesterol and leptin and cholesterol categories in male smoking and non-smoking patients with DM-type-1 and DM-type-2. Considering the cholesterol categories, subjects indicated that all normal non-smoking subjects were in the limit of desirable cholesterol level. Smoking control group showed about 81% subjects in category of desirable cholesterol level. About half (55%) of the patients in non-smoking diabetic group were in the category of high cholesterol and showed highly significant involvement of plasma cholesterol ($p < 0.001$). About 87% smoking diabetics were in the category of high cholesterol and showed highly significant variations while compared with smoking controls ($p < 0.001$). The comparison of the leptin levels for patients with diabetes type 2 vs normal control subjects and DM-type-1 and DM-type-2 showed highly significant change. For the plasma protein, all comparisons showed non-significant variations. Reports on the precise involvement of plasma leptin in diabetics, smokers and diabetic smokers, however, are scarce. The future studies hence, might verify the influence of leptin in the pathogenesis of diabetes and smoking as investigated in the current study. Conclusively, the present data of both patient groups with diabetes pertains interesting information regarding future exploration of the pathogenesis and therapeutic advancement in the area of diabetology.

INTRODUCTION

It is known that the cigarette smoking may increase risk for type 2 diabetes mellitus (DM) (van Dam, 2003). However, the effect of smoking on the pathogenic factors for the development of diabetes is little explored (Massulli *et al.*, 2006). It was suggested that in diabetes care, smoking cessation is of utmost importance to facilitate glycemic control and limit the development of diabetic complications (Elliasson, 2003). It was shown that cigarette smoking is an important modifiable risk factor of type 2 diabetes particularly in men from the general population (Meisinger *et al.*, 2006). Several prospective epidemiological studies of diabetes incidence in men have included cigarette smoking as a possible risk factor (Medalie *et al.*, 1975; Kawakami *et al.*, 1997).

It is notable that most of those mentioned studies included smoking as one variable significantly involved in diabetes. However, further studies are needed to enhance our understanding of the relationship between diabetes and smoking. For the present study, the major purpose was to investigate the independent effects of smoking on the development of DM-type-2 in men.

The accumulation of visceral fat in conditions associated with insulin resistance (Kawakami *et al.*, 1997) is an important risk factor in diabetes and atherosclerosis. The role of adipose tissue and specifically the adipocytokines play role in the development of insulin resistance leading to diabetes and related disorders (Matsuzawa *et al.*, 1999). Leptin and adiponectin are concerned with insulin sensitivity and diabetes (Caro *et al.*, 1996; Maeda *et al.*, 1996; Shimomura *et al.*, 1999). Furthermore, diabetes, hypertension, and metabolic syndrome have been studied and linked to each other clinically (Sohail Ashraf *et al.*, 2006; Basir and Sohail Ashraf, 2007; Sohail Ashraf and Basir, 2007). Blood cholesterol and cytokines (Hussain *et al.*, 2007a,b; Sohail and Hussain, 2013) have been considered important in patients with diabetes.

In view of the controversial results obtained by different investigators, we needed to determine the blood cholesterol and leptin in DM-type-1 and DM-type-2. This information, in turn, may help us understanding the clinical and biochemical background of the pathophysiology and proper management of diabetes. In general, the data of both patient groups- DM-type-1 and DM-type-2- in the present work via leptin and cholesterol determination may provide interesting information for the future exploration of the pathogenesis and therapeutic advancement in the area of diabetology.

The significance of the present investigation is to explore the role of smoking via determining the plasma leptin levels and cholesterol concentrations and categories in diabetes mellitus. This approach may provide opportunity in future to understand the influence of characteristic features common in smoking and diabetes. Furthermore, the variations in leptin levels and cholesterol categories/ concentrations may provide us valuable information about the mechanisms involved in diabetes and smoking. The major significance of the present project, however, is that it might help developing new therapeutic products with the additional knowledge

obtained from the pathophysiological association between cholesterol and leptin levels in smoking and non-smoking subjects having diabetes mellitus.

MATERIALS AND METHODS

Male Patients with diabetes mellitus were diagnosed properly and general clinical and biochemical evaluations were carried out. The sample size in the respective populations was based upon the selected confidence limit (95% confidence interval) that is usually preferred in biomedical studies. A part of the data of the patients with smoking and non-smoking DM-type-1 and DM-type-2 was further designed by splitting in specific groups considering cholesterol levels.

Male subjects with the same age range were selected for the present study. This comprised normal non-smoking subjects, smoking controls and non-smoking and smoking diabetics. The patients filled a questionnaire about their smoking habits, general health issues, and a possibility of having latent form of diabetes.

Selection of the patients with diabetes was carried out by the detailed information obtained by questionnaire. The data of the smoking and non-smoking diabetic patients was obtained and only those patients using 3-5 cigarettes per day for at least three months were taken for the present study.

The desirable, borderline high, and high levels of blood cholesterol in mg/dl were followed in the present study considering standard reports. Subjects in cholesterol categories were determined and converted into percent values for comparison purposes. Questionnaire was employed for investigating the smoking habit of the subjects. Fasting glucose, triglycerides, total and HDL cholesterol, and blood pressure were also measured for diagnostic purpose. Other biochemical investigations involving plasma levels of leptin, total protein and cholesterol were carried out using ELISA kits and standard methods.

RESULTS AND DISCUSSION

The estimation of plasma levels of cholesterol, total protein and leptin was compared among four subject groups. On the basis of cholesterol levels, cholesterol categories were constructed that indicated that all normal non-smoking subjects were in the limit of desirable cholesterol level and hence the subjects taking part as normal controls in the present study were considered as healthy non-smoking controls (Table 1). Smoking control group showed about 81% subjects in category of desirable cholesterol level, whereas the remainders (about 9% each for borderline high and high cholesterol levels) were other categories. Hence, this clearly showed non-significant variation when compared with the categories in healthy non-smoking controls ($p > 0.05$; Table 1).

The data of non-smoking diabetic group showed scattered cholesterol values from desirable to borderline high to high categories. About half (55%) of the patients in this group were in category of high cholesterol and showed highly significant involvement of plasma cholesterol ($p < 0.001$; Table 1). About 87% smoking diabetics were in the category of high cholesterol and showed highly significant variations while compared with smoking controls ($p < 0.001$; Table 1).

Table 1. Subject categories considering cholesterol blood levels in male smoking and non-smoking diabetics.

Subject groups	Categories of the subjects				Cholesterol (mean \pm SEM; mg/dl)	Two-tailed p value (unpaired t-test)
	Desirable	Borderline high	High	Total subjects (n=150)		
Healthy non-smoking controls	32 (100%)	Nil (0%)	Nil (0%)	32 (100%)	189.99 \pm 11.20	
Smoking controls	26 (81.25%)	03 (9.375%)	03 (9.375%)	32 (100%)	219.75 \pm 10.72	> 0.05 (Smoking controls Vs Healthy non-smokers)
Non-smoking diabetics	08 (20%)	10 (25%)	22 (55%)	40 (100%)	252.23 \pm 13.16	<0.001 (Non-smoking diabetics Vs Healthy non-smokers)
Smoking diabetics	03 (6.52%)	03 (6.52%)	40 (86.96%)	46 (100%)	263.27 \pm 7.37	<0.001 (Smoking diabetics Vs Smoking controls)

The comparison for patients with diabetes type 2 vs normal control subjects and diabetes type 1 vs type 2 showed highly significant change in leptin levels (Tables 2 and 3). However, no significant variation in leptin levels could be found for diabetes type 1 patients vs normal controls (Tables 2 and 3). For the plasma protein, all comparisons showed non-significant variations (Tables 2 and 3).

The current study via determining the blood levels of cholesterol and leptin shows the involvement of smoking in diabetes mellitus (Tables 1, 2 and 3). The positive relationship between cigarettes smoked and diabetes incidence has been noted in other prospective studies (Rimm *et al.*, 1993; Rimm *et al.*, 1995).

It is important to note that in view of a relatively small number of epidemiological studies assessing the influence of smoking frequency and intensity on diabetes, showing other methodological limitations of these studies and our lack of understanding of how cigarette smoking might influence diabetes, we need additional evidence. To clarify a causal association, future research will need to address these important issues. This might be carried out by studying precisely the influence of a variety of biochemical and physiological changes e.g. blood cholesterol. The analysis of the present data is helpful as it showed that the order of the cholesterol level is: Smoking Diabetics > Non-Smoking Diabetics > Smoking Controls > Healthy Non-Smoking Controls. These results provide an idea of the influence of blood cholesterol in smokers, and smoking subjects with diabetes. Such other future studies can lead us to predict about multifactorial analysis of variations. To have better understanding about the association of cigarette smoking and diabetes, it has been suggested to perform future studies considering several required criteria e.g. consistency of findings, having a plausible biological mechanism, and evidence that one has ruled out other possible explanations (Evans, 1976).

A study shows that nonsmokers experienced higher incidence of diabetes than smokers (Shaten *et al.*, 1993), which supports the presumption that the selection criteria led to an initially higher risk for diabetes in this particular group of nonsmokers. This does not suggest, however, that nonsmokers in general are at higher risk for diabetes than smokers; indeed, prospective and laboratory studies show that smoking may increase the risk for diabetes (Facchini *et al.*, 1992; Rimm *et al.*, 1995). These studies are in accordance with our present results. The patients with type 2 diabetes have a two-to four-fold greater risk of cardiovascular mortality than non-diabetic individuals (Nesto, 2001). In order to prevent coronary events in the diabetic population, it is important to treat modifiable cardiovascular risk factors. Like the present study, investigating the role of cholesterol in diabetes and smoking, plasma cholesterol levels and other related parameters might be evaluated to know whether cigarette smoking is significant predictor of cardiovascular disease mortality in men with and without diabetes. Several other criteria should be met before seriously entertaining the notion that cigarette smoking is a cause of diabetes.

Table 2. Concentrations of leptin and total protein blood levels in male diabetic patients.

Subject groups	Subjects (total =93)	Leptin (ng/ml)	Total protein (mmol/l)
NC	24	10.8 ± 2.2	68.4 ± 6.7
T1-DM	32	9.9 ± 2.3**	68.8 ± 6.7
T2-DM	37	13.5 ± 2.8*	66.6 ± 6.9

The values are Mean ± S.D.; T1-DM and T2-DM respectively are for diabetes mellitus type-1 and diabetes mellitus type-2; NC are the normal control subjects; *NC vs T2-DM showed p= 0.0002; **T1-DM vs T2-DM showed p= 0.0001; Unpaired t-test was used for obtaining two-tailed p values, The detailed statistical analysis is given in Table 3.

Table 3. Statistical analysis for leptin and total protein blood levels in male diabetic patients.

Subject groups for leptin and total protein estimations		Statistical analysis (unpaired t test)			
		df	t-value	SE of difference	Two-tailed p-value
NC vs T1-DM	Leptin (ng/ml)	54	1.4761	0.610	0.1457
	Total protein (mmol/l)	54	0.2211	1.809	0.8259
NC vs T2-DM	Leptin (ng/ml)	59	3.9886	0.677	0.0002*
	Total protein (mmol/l)	59	1.0066	1.788	0.3182
T1-DM vs T2-DM	Leptin (ng/ml)	67	5.7785	0.623	0.0001*
	Total protein (mmol/l)	67	1.3386	1.644	0.1852

The T1-DM and T2-DM respectively denote diabetes mellitus type-1 and diabetes mellitus type-2; NC are the normal/healthy control subjects; * highly significant

The present report presents the view that smoking via changing the plasma cholesterol is a major risk factor for patients with diabetes mellitus. The data explains that normal healthy men as well as men with diabetes (non-smoking) present comparatively low concentrations of plasma cholesterol. However, an important question still remains to be solved that if plasma cholesterol varies significantly in both smoking and non-smoking diabetic men as well as smoking men without diabetes, whether plasma cholesterol change occurs more as a result of smoking, diabetes or other characteristics involved partly with both of these conditions.

Association between plasma leptin and diabetes has been investigated (Sari *et al.*, 2010 and Bandaru and Shankar, 2011). With that context, our current study provides further evidence that leptin levels significantly increase in diabetes mellitus type 2. Data on the precise involvement of plasma leptin in diabetics, smokers and diabetic smokers, however, is scarce. The future studies might uncover the influence of leptin and other adipocytokines in the pathogenesis of diabetes and smoking. Hence, in general, the data of both patient groups- with type 2 and type 1 diabetes mellitus- in the present study provides interesting information for the future exploration of the pathogenesis and therapeutic advancement in the area of diabetology.

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