Original article

Assessment of oxidative damage and lipid profile levels in tobacco chewers with Type 2 diabetes mellitus

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Abstract

Objectives: To assess oxidative damage and lipid profile levels in Type 2 diabetes mellitus patients with or without tobacco chewing habit. ***Methods:*** Total of 141 newly diagnosed Type 2 diabetes mellitus patients with tobacco chewing habit from past >18 years were included, 136 Type 2diabetes mellitus without any tobacco chewing habit, 140 normal healthy subjects without any tobacco chewing habit were included in the study. Blood sample was collected after 8-12 hours fasting from each subject to estimate glucose and cholesterol. The plasma was used for estimation of total antioxidant (TAA) activity, Vitamin C and thiobarbituric acid reacting substances (TBARS) by standard methods. Statistical analysis was done with SPSS version 16. ***Results:*** The glucose, cholesterol and TBARS levels were found to be increased significantly ($p<0.001$) in diabetic patients with tobacco chewing habit, as well as TAA, Vitamin C levels decreased significantly ($p<0.001$) when compared with healthy individuals. ***Conclusion:*** We observed increased oxidative stress and declined levels of antioxidants in newly diagnosed type 2 diabetes patients with tobacco chewing habit. It is known that hyperglycemia induces oxidative stress and further facilitate the progression of diabetic complications.

Keywords: Tobacco chewing; oxidative stress; antioxidants; hyperglycemia; Type 2 DM

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Introduction

Chewing tobacco is a major risk factor for lifestyle disorders. Some common chewing tobacco products available in Nepal are betel-quid; khaini, gutka and zarda[1-3]. Tobacco used along with betel-quid contain the chemical substances like polyphenols, alkaloids, some of the trace elements and free radicals[4]. Khaini is also known as surti in Nepal. It includes a mixture of slaked lime and dried tobacco leaves[2]. Gutka containing powdered tobacco along with areca nut, slaked lime catechu and other sweetening substance[5]. Zarda is powdered tobacco available in packets [3].

It was estimated that 4.1 deaths per day are due to diseases caused by tobacco consumption[6]. Smokeless tobacco produces high levels of nicotine, which is the prime cause for oral cancer and acute cardiovascular disorders[7]. Higher prevalence of diabetes is due to long-term use of smokeless tobacco[8].Direct consumption of tobacco is associated with insulin resistance[9]. Insulin resistance is the major cause for the development of dyslipidemia, obesity, hypertension, type 2 diabetes mellitus (T2DM) and other cardiovascular disorders[10]. Smokeless tobacco increases the total cholesterol, triglycerides and lower high-density lipoprotein levels[11].

Tobacco and their products are rich in pro-oxidants are proved to induce oxidative stress. Reactive oxygen species produced by tobacco is one of the causes of beta cell dysfunction, protein damage and impairs endothelial function[12].Antioxidants have a protective role against the oxidative stress. The balance between reactive oxygen species and antioxidants is disturbed; it leads to increased oxidative stress. Mutagens present in tobacco are

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capable of initiating and promoting oxidative damage to DNA[13].The objective of the study is to assess the oxidative damage and lipid profile levels in T2DM patients with or without tobacco chewing habit from Pokhara, Nepal.

Methods

It was a case and control study, included a total of 141 newly diagnosed T2DM patients with tobacco chewing habit from past >18 years. 136 T2DM without any smoking habit, 140 normal subjects without any smoking habit and diseases were involved in the study. Detailed history includes age, sex, occupation; smoking and drinking habits of the patients were collected with the help of a proforma. ***Exclusion criteria:*** Diabetic patients with other illness, complications and on multivitamin supplementation were excluded from the study. None of the healthy volunteers were taking medication that would influence the antioxidant and oxidative stress level.

Sample collection and analysis

Fasting blood samples were collected into EDTA bottles from each subject. Plasma was collected by centrifugation at 3000 rpm and stored at 20˚C for analysis within 24 hours. Glucose and lipid profile was estimated by kit method (Randox laboratories) and processed through semi auto-analyzer (Microlab 300). Lipid profile includes total cholesterol (TC), triglycerides (TG), very low- density lipoproteins (VLDL), Low-density lipoproteins (LDL) and high-density lipoproteins (HDL). Total antioxidant activity (TAA)[14], α-tocopherol [15], vitamin C[16] and thiobarbituric acid reacting substances (TBARS)[17] were estimated by standard methods.

The results were reported as the mean and standard deviation (SD). The statistical analysis was done with SPSS 16 version software. Data were analyzed by using Independent Sample t-test, one-way ANOVA followed by Bonferroni's *post-hoc* test. P value ≤ 0.05 was expressed as significant at 95% confidence intervals.

Ethical clearance was obtained from institutional research committee. Informed consent was taken from all the subjects. This study was conducted in Manipal Teaching Hospital, Pokhara, Nepal.

Results

There was a significant evidence ($p<0.001$) of lipid peroxidation in the T2DM patients (6.21±0.89 nmol/ml) compared with controls and T2DM without any tobacco chewing habit. There was also a significant decrease ($p<0.001$) in the activity of Vitamin C, α- tocopherol and TAA. Moreover, enhanced peroxidative stress as seen by increased TBARS along with significant depletion of nutritive antioxidants. (Table 1)

Table :1 Oxidative stress, antioxidant levels in controls, T2DM with tobacco chewing and without tobacco chewing habit

Parameters	Controls (N=140)	T2 DM with tobacco chewing habit (N=141)	T2 DM without tobacco chewing habit (N= 136)
TBARS (nmol/ml)	1.52±0.75	6.21±0.89*	3.52± 0.14#$
Vitamin C (mg/dl)	0.7±0.43	0.2±0.11*	0.35±0.16#$
α- tocopherol (mg/dl)	1.02±0.32	0.48±0.1*	0.62±0.12#$
TAA (µmol/L)	934±165	512±117*	604±184#$

*P <0.001 Control Vs T2DM with tobacco chewing habit

# P <0.001 Control Vs T2DM without tobacco chewing habit

$ P <0.01T2DM with tobacco chewing habit Vs T2DM without tobacco chewing habit

The mean TC, TG, VLDL, LDL and glucose levels were increased significantly (P<0.001) in T2DM with tobacco chewing habit compared to T2DM without tobacco chewing habit and control. HDL was significantly decreased (P<0.001) in T2DM with tobacco chewing habit compared to T2DM without tobacco chewing habit and control. Atherogenic index (AI) was increased significantly (P<0.001) in T2DM with tobacco chewing habit compared to T2DM without tobacco chewing habit and control (Table 2). These findings were strengthened by ANOVA test (Table 3).

Table :2 Lipid profile levels in controls, T2DM with tobacco chewing and without tobacco chewing habit

Parameters	Controls	T2 DM with tobacco chewing habit	T2 DM without tobacco chewing habit
Age	47.82±19.2	50.56 ± 22.2	55.2± 8.92
TC mg/dl	172± 10.4	211 ± 34.3*	182 ± 5.1#$
TAG mg/dl	100 ± 22.9	214 ±26.8*	146 ± 18.9#$
VLDL mg/dl	20.1± 4.5	42 ± 5.2*	29 ± 3.7#$
LDL mg/dl	99 ± 13.7	139 ± 35.5*	120 ± 8.3#$
HDL mg/dl	53.6 ± 8.1	28.9± 5.9*	32 ± 4.9#$
AI	0.18±0.04	0.8± 0.06*	0.57± 0.02#$
Glucose mg/dl	87 ±12.9	188 ±38.9*	160 ±1.2#$

*P<0.001 Control Vs T2DM with tobacco chewing habit
# P <0.001 Control Vs T2DM without tobacco chewing habit
$P <0.001 T2DM with tobacco chewing habit Vs T2DM without tobacco chewing habit

Table 3: Statistical analysis by ANOVA test analysis of variance LDL and HDL

Parameter		Sum of Squares	df	Mean Square	F	Sig.
LDL	Between Groups	102358.410	2	51179.205	84.320	<0.001
	Within Groups	251284.019	414	606.966		
	Total	353642.429	416			
HDL	Between Groups	26429.547	2	13214.774	313.232	<0.001
	Within Groups	17465.997	414	42.188		
	Total	43895.544	416			

Discussion

Chewing tobacco is a major risk factor for lifestyle disorders. Tobacco chewing products contain high levels of N-nitrosamino acids, N-nitrosonornicotine (NNN) and 4-(methylnitrosamino)-1 (3-pyridyl)-1 butanone (NNK)[18] were reported in *khaini, zarda*and *gutka* were higher than the permitted limits. Tobacco is rich in pro-oxidants more toxic than nicotine, is proved to induce oxidative stress, beta cell damage and apoptosis among chronic tobacco chewers[19]. Tobacco not only increases oxidative stress but also causes insulin resistance[20]. In our study oxidative stress was increased significantly in T2DM with tobacco chewing habit compared to controls. Our results are correlating with previous studies, reported elevated levels of TBARS in T2DM patients chewing smokeless tobacco[21-24]. Free radicals are attributed to playing a major role, which is further exacerbated by the weak antioxidant defense in T2DM leading to the development of microvascular and macrovascular complications. We observed decreased levels of TAA, vitamin C and E in tobacco chewers with T2DM as compared to controls. Our results are supported by Shrestha et al. reported significantly decreased the activity of vitamin C and E in users of pan masala containing tobacco[25]. TAA is the total non-enzymatic antioxidant activity. The normal range of the total antioxidant activity by this method is 600-1600 µmol/L[15]. Our data revealed that low levels of TAA in T2DM when compared to other groups, these results are suggesting that the patients with tobacco chewing habit should consume more antioxidant to maintain redox balance.

TC, HDL, TG was determined enzymatically using standard kit methods. LDL level was estimated by using Friedewald's equation: LDL=TC−HDL−VLDL (VLDL = TGs/5)[26]. Atherogenic index (AI) was calculated as log (TG/HDL)[27]. In our study there were elevated levels of TC, TAG, LDL and reduced HDL in T2DM with tobacco chewing habit. These findings are correlated with previous authors[28,29]. Contrary to these results others have failed to find the association between lipid profile levels[30]. AI is a strong indicator to predict the risk of atherosclerosis and coronary heart disease[27,31]. It reflects the true relationship between protective and atherogenic lipoprotein and is associated with the size of pre- and anti-atherogenic lipoprotein particle. AI value is below 0.11 indicating the low risk of CVD; the values between 0.11 to 0.21 and greater than 0.21 are related to intermediate and high risks, respectively [32]. Lipid peroxide compounds enhance the synthesis of prostaglandins which further produces free radicals. Finally, all the pathways increase the production of nitric oxide, which is a well-known risk factor for atherosclerosis.

In our study we have observed the increased levels of glucose than T2DM without chewing habit reduced insulin sensitivity and development of insulin resistance has been associated with tobacco consumption. Tobacco increases the circulating anti-insulin hormones like cortisol, catecholamines and growth hormone, which will increase glucose levels[33]. We did not find any significant changes between male and female of the same group.

Conclusion

These results are indicating that tobacco chewing is associated with the increased TC, TGs, LDL, glucose, oxidative stress, declined levels of HDL and antioxidants. It is known that hyperglycemia, hyperlipidemia induced oxidative stress to facilitate the progression of organ dysfunctions and diabetic complications. Future studies should aim to monitor oxidant and enzymatic antioxidant status as well as genetic susceptibility of tobacco chewers might be useful in predicting the risk of T2DM

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Authors Contributions:

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