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Analysis of related factors of Diabetic Retinopathy in Patients with Newly diagnosed type 2 diabetes：Heavy smoking may be an important risk factor. (words 2470, tables 4, figures 2)

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Abstract

Background: Cigarette smoking is a known risk factor for many diseases, including various kinds of cancer and cardiovascular diseases. 20% of newly diagnosed type 2 diabetic (T2DM) patients are diagnosed with DR. The explanation of this phenomenon is often that the onset of T2DM is insidious, and patients may have a long history before diagnosis. We investigated the related factors of DR, and explore the correlation between smoking and DR in patients with newly diagnosed type 2 diabetes who visited in the hospital from December 2018 to April 2019.

Methods: Finally 947 patients enrolled in the study. According to whether they were diagnosed with DR, patients were divided into two groups (diabetic retinopathy group and non-diabetic retinopathy group). Calculate smoking index and assess smoking severity. The factors such as sex, age, hypertension, proportion of patients with DM diagnosed age <40 years old, family history of diabetes, drinking history, HbA1c, BMI and smoking status were compared between two groups, and logistic regression was used on analyzing the relationship between DR and the factors above.

Results: There was no statistically significant difference between two groups in sex, age, hypertension, proportion of patients with DM diagnosed age <40 years old, family history of diabetes, drinking history and HbA1c. BMI was significantly higher in DR patients(27.7±4.2vs26.7 ±4.4, p=0.004). Smoking status was different between the two groups(χ^2=6.350, p=0.042). BMI is a related factor for DR in newly diagnosed diabetic patients (OR=0.634, p=0.012)by logistic regression analysis. When BMI was ≥28 kg/m^2, heavy smoking was significantly associated with DR(OR=2.336, p=0.027).

Conclusions: Heavy smoking is an independent risk factor for DR in patients with newly diagnosed DM. And higher smoking amount also maybe a risk factor of getting DR. Therefore, to elucidate the correlation clearly, we need to do a long-term cohort study with large sample to confirm.

Keywords: Smoking index, Type 2 diabetes mellitus, Diabetic retinopathy, newly diagnosed diabetes mellitus.

Strengths and limitations of this study

Current studys on the effect of smoking on diabetic retinopathy have been inconsistent.

20% of newly diagnosed type 2 diabetes mellitus(T2DM) are diagnosed with diabetic retinopathy(DR). The explanation is that the onset of T2DM is usually insidious, and many patients with diabetes have been suffering from diabetes for years before being diagnosed. There are few studies on the factors related to DR in newly diagnosed type 2 diabetes patients.

Unlike most other studies, we firstly evaluated the effect of smoking on diabetic retinopathy according to smoking index in newly diagnosed diabetic patients. We found that heavy smoking may be a risk factor for DR in patients with newly diagnosed diabetes with obesity(OR=2.336, p=0.027).

Although our department is responsible for the first diagnosis and identification of diabetes in Tianjin, it is still unable to timely diagnose those patients who just got diabetes.

1. Introduction

Diabetes mellitus (DM) is a chronic metabolic disease characterized by hyperglycemia caused by various etiologies, and the incidence is increasing year by year worldwide. According to the report of the International Diabetes Federation (IDF) in 2017 about 425 million people were affected by DM, and the number of the patients is expected to reach 629 million by 2045[1]. China was the country with the highest number of diabetes patients, and the prevalence has increased from 0.67% in 1980 to 10.4% in 2013[2].A meta-analysis based epidemiological studies conducted from 35 research centers around the world showed that the prevalence of diabetic retinopathy(DR)was 35.4%[3]. DR is a highly specific microvascular complication caused by diabetes, and its prevalence is significantly correlated with the course of DM and blood glucose levels[4]. DR is the most important reason of irreversible blindness in working-age adults. Chronic hyperglycemia causes not only retinal vascular diseases, but also leads to damage of retinal neurons, both of those two factors above lead to vision loss[5].20%[3] of newly diagnosed type 2 diabetic (T2DM) patients are diagnosed with DR. The explanation of this phenomenon is often that the onset of T2DM is insidious, and patients may have a long history before diagnosis. Are there any other risk factors for DR in these newly diagnosed patients? There is no relevant research report at present.

Smoking is harmful and it's also an important risk factor for cardiovascular diseases, cerebrovascular diseases, respiratory diseases and malignant tumors. Smoking is one of the most serious public health issues across the world and it is responsible for about 7 million deaths worldwide per year[6].In a 14-year prospective cohort study Jee SH found that people smoking more than 20 cigarettes a day had a 1.55-fold higher incidence of diabetes than a non-smoker[7] . We all know that smoking is harmful, and smoking may cause the presence of protein in the urine increasing. We all expect that smoking has a promoting effect on DR, but we have no direct clinical epidemiological evidence that smoking has an effect on DR in these newly diagnosed patients[8]. Many diabetic patients have started smoking before they diagnosed with diabetes. Does smoking contribute to the complications of DR in newly diagnosed patients? Little research has been done on the relationship between smoking and DR in newly diagnosed diabetic patients. In our present research, we investigated the related factors of DR, and explore the correlation between smoking and DR in patients with newly diagnosed type 2 diabetes who visited in the hospital from December 2018 to April 2019.

2. Research design and methods

2.1. Participants

Study participants were type 2 diabetes patients who visited the Metabolic Disease Management center of the Tianjin 4th Central Hospital from December 2018 to April 2019. This clinical study protocol was approved by the institutional review board of Tianjin 4th Central Hospital, and all steps were conducted in accordance with the principles the World Medical Association Declaration of Helsinki[9]. (Trial registration code: ChiCTR1900020728).

2.2. Inclusion criteria

Our study participants enrolled at baseline with newly diagnosed with T2DM within past six months; patients who could communicate independently and describe their smoking status; none of the smokers had given up smoking before the study; all patients underwent diabetic retinopathy screening in the study.

2.3. Exclusion criteria

Exclusion criteria : Type 1 diabetes and other specific types of diabetes; acute complications of diabetes, such as diabetic ketoacidosis or lactic acidosis; hypovolaemic; subjects with diabetic nephropathy complications, rheumatic diseases, autoimmune diseases ,pregnancy, malignant tumor, infection, foot ulcer, mental illness, thyroid dysfunction, severe hepatic insufficiency or renal insufficiency , anemia, heart failure or respiratory insufficiency; cognitive impairment, and communication disorders.

2.4. Evaluation of Clinical variables

Information about patients' names, sex, phone numbers, Body mass index (BMI), age, smoking history, drinking history, family history of DM, hypertension and HbA1c was collected by using a uniform information table. The formula for BMI is weight in kilograms divided by height in meters squared.BMI (≥28kg/m^2) met obesity criteria according to Chinese standards. HbA1c levels were measured with affinity chromatography. Smoking status assessment: according to the WHO (1984) smoking survey method, smoking at least one cigarette per day for more than one year is considered as smoking history. The smoking index is calculated by multiplying the number of cigarettes smoked per day by the number of years the person has smoked. In our study, according to the smoking index, 0 means non-smoking, ≤500 means light and moderate smoking, and > 500 means heavy smoking. According to the age of diagnosis of T2DM, subjects were divided into two cases(≥40 or <40 years old)[10].

According to EURODIAB guidelines, screening for DR was carried out with one 45° field retinography, centred on the fovea. If DR was suspected, another two retinographies of 45° were taken[11]. Ophthalmologists judged whether the patient had DR or not according to the results of the funduscopic examination and guidelines. In this study, there were only two cases

according to the results (diabetic retinopathy DR and non-diabetic retinopathy NDR), and the patients were divided into two groups (DR group and NDR group).

2.5. Statistical analysis

Statistical analysis was performed using SPSS 16.0 software. We concluded that a significant difference exists if P<0.05 (two-sides) . For continuous variables, Kolmogorov-Smirnov Z-test was used to check . Once normal distribution and homogeneity of variance were satisfied, values were represented by the mean±SD and student t-test was used to compare the difference between two groups. Otherwise, values were represented by the median and rank sum test was applied. For categorical variables, chi-squared test was performed. The occurrence of DR was identified as the dependent variable, and the related risk factors were listed as independent variables. Both of the variables were included in a multivariate logistic regression analysis. α=0.05.

3. Results

3.1. Baseline characteristics of participants with newly diagnosed T2DM

The clinical trial flow chart is shown in Figure 1. Initially, 952 patients entered the study and 5 patients were unable to see the fundus. Finally, a total of 947 patients were enrolled in the study, including 566 males (59.8%) and 381 females (40.2%). The mean ages were 53.2±11.6 years. The average BMI was 27.5±4.3kg/m^2. 430 patients (45.4%) have been smoking. The average smoking age was 27.8±12.2 years (2~60 years). They smoked 21.8±12.3 cigarettes per day. 147 patients(15.5%) were diagnosed T2DM before 40 years old . There were 580 patients (61.2%) with family history of DM, and 195 patients(20.6%) with drinking history. The mean HbA1c was 9.53±2.13%. There were 755 NDR patients (79.7%) and 192 DR patients (20.3%). 12 patients (1.3%) suffered from proliferative diabetic retinopathy(PDR). Baseline characteristics were shown in Table 1.

3.2. The comparison of clinical data between DR and NDR groups was shown in Table 2.

There was no statistically significant difference between the two groups in sex, age, hypertension, proportion of patients with DM diagnosed age <40 years old, family history of diabetes, drinking history and HbA1c. BMI was significantly higher in DR patients(27.7±4.2vs26.7±4.4, p=0.004). In our study, according to the smoking index, 0 means non-smoking, ≤500 means light and moderate smoking, and > 500 means heavy smoking. We found that smoking status was different between the two groups(χ^2=6.350, p=0.042).

3.3. The occurrence of DR was identified as the dependent variable, and the related risk factors were listed as independent variables. Both of the variables were proceeded through a

multivariate logistic regression analysis in Table 3. BMI(≥28kg/m^2) is a related factor for DR in newly diagnosed diabetic patients (OR=0.634, p=0.012).

3.4. The average age of newly T2DM patients with obesity was 50.4±12.4 years, while that of patients without obesity was 55.1±10.6 years. The former was significantly lower than the latter(t=6.094, p<0.001). Because the onset of T2DM is insidious, patients may have a long history before diagnosis. We divided the participants into two parts according to whether BMI was ≥ 28 kg/m^2. We compared the incidence of DR in patients with different smoking status(Figure 2), and used logistic regression analysis to analysis the related factors of DR (Table 4). When BMI was ≥28 kg/m^2, we found that heavy smoking was significantly associated with DR(OR=2.336, p=0.027).

Table 1 Baseline characteristics of participants with newly diagnosed T2DM.

Characteristics of participants	Comment	Statistical value
Sex（n，%）	Male	566（59.78%）
Age（years）		53.2±11.6
Family history of DM（n，%）		580（61.2%）
Age of diagnosis of DM（n，%）	< 40 years old	147（15.5%）
HbA1c（%）		9.53±2.13
BMI（kg/m^2）		27.5±4.3
Hypertension（n，%）		498（52.6%）
Drinking history		195（20.6%）
Smoking status（n，%）	Non-smoking	517（54.6%）
	Light and moderate smoking	212（22.4%）
	Heavy smoking	218（23.0%）
The number of years a person has smoked（years）		27.8±12.2
DR（n，%）		192（20.3%）
PDR（n，%）		12（1.3%）

Table 2 Comparison of clinical data between DR and NDR groups

Items		NDR (n=755)	DR (n=192)	t/χ^2	P value
Sex	Male	447（79.0%）	119（21.0%）	0.490[a]	0.510
	Female	308（80.8%）	73（19.2%）		
Age	years	53.3±11.7	52.9±11.1	0.350[b]	0.726
Family history of DM	no	288（78.5%）	79（21.5%）	0.580[a]	0.456
	yes	467（80.5%）	113（19.5%）		
Drinking history	no	608（80.9%）	144（219.1%）	2.863[a]	0.109
	yes	147（75.4%）	48（24.6%）		
Hypertension[c]	no	349（81.2%）	81（18.8%）	1.457[a]	0.265
	yes	392（78.7%）	106（21.3%）		
DM diagnosed age <40	no	123（83.7%）	24（16.3%）	1.678[a]	0.220
	yes	632（79.0%）	168（21.0%）		
HbA1c	%	9.51±2.13	9.60±2.12	-0.546[b]	0.585
BMI	kg/m^2	27.7±4.2	26.7±4.4	2.921[b]	0.004
Smoking history	no	424(82.0%)	93(18.0%)	3.682[b]	0.062
	yes	331(77.0%)	99(23.0%)		
Smoking status	Non-smoking	424（82.0%）	93（18.0%）	6.350[a]	0.042
	Light and moderate smoking	170（80.2%）	42（19.8%）		
	Heavy smoking	161（73.9%）	57（26.1%）		

Note: [a] chi-square test , [b] student t test and [c]19 cases of patients with missing data. There was no

statistically significant difference if p >0.05.

Table 3 Logistic regression analysis of related factors for DR

Independent variables	B	SE	Wald χ^2	P value	OR	95%CI
Sex	0.085	0.224	0.144	0.705	1.089	0.702-1.688
Hypertension	0.171	0.172	0.993	0.319	1.187	0.847-1.663
Drinking history	0.178	0.219	0.659	0.417	1.195	0.778-1.835
HbA1c	0.075	0.168	0.197	0.657	1.077	0.775-1.498
BMI	-0.456	0.181	6.334	0.012	0.634	0.445-0.904
Family history of DM	-0.125	0.170	0.547	0.460	0.882	0.633-1.230
DM diagnosed age <40 years	0.011	0.263	0.002	0.966	1.011	0.604-1.692
Smoking status			3.479	0.176		
Light and moderate smoking	0.087	0.249	0.121	0.728	1.091	0.669-1.777
Heavy smoking	0.427	0.244	3.056	0.080	1.533	0.950-2.474
Constant	-1.579	0.631	6.259	0.012	0.206	

Note: assignment instructions as follows---Sex (male 1, female 2); BMI(<28 0, ≥28 1); HbA1c(≤9% 0, >9 1); Hypertension、drinking history、family history of DM and DM diagnosed age <40 years no 0, yes 1; Smoking status non-smoking 0, light and moderate smoking 1, heavy smoking 2, non-smoking as the reference.

Table 4 Logistic regression analysis of related factors for DR(BMI<28kg/m^2, ≥28kg/m^2)

	Independent variables	B	SE	Wald χ^2	P value	OR	95%CI
BMI<28 kg/m^2	Sex	-0.167	0.283	0.347	0.556	1.182	0.678-2.060
	Hypertension	0.070	0.212	0.107	0.743	1.072	0.707-1.625

	Drinking history	0.059	0.275	0.046	0.830	1.061	0.619-1.817
	HbA1c	-0.025	0.205	0.015	0.902	0.975	0.653-1.457
	Family history of DM	-0.368	0.204	3.247	0.072	0.692	0.464-1.033
	DM diagnosed age <40 years	0.099	0.380	0.068	0.794	1.104	0.525-2.324
	Smoking status			1.037	0.595		
	Light and moderate smoking	0.278	0.318	0.766	0.382	1.321	0.720-2.506
	Heavy smoking	0.295	0.318	0.858	0.354	1.343	0.950-2.474
BMI≥28 kg/m^2	Sex	-0.009	0.382	0.001	0.980	0.991	0.468-2.096
	Hypertension	0.345	0.313	1.220	0.269	1.412	0.765-2.607
	Drinking history	0.465	0.377	1.526	0.217	1.592	0.761-3.331
	HbA1c	0.335	0.304	1.213	0.271	1.398	0.770-2.536
	Family history of DM	0.449	0.325	1.905	0.167	1.566	0.828-2.962
	DM diagnosed age <40 years	0.046	0.376	0.015	0.902	1.047	0.501-2.187
	Smoking status			7.156	0.028		
	Light and moderate smoking	-0.173	0.422	0.169	0.681	0.841	0.368-1.922
	Heavy smoking	0.848	0.384	4.879	0.027	2.336	1.100-4.960

4. Discussion

DR is a serious chronic microvascular complication of DM. In the early stage of DR, patients may have only mild symptoms or no symptoms. With progression of the disease, new blood vessels formed in the areas of capillary non-perfusion in the fundus, which may eventually lead to blindness. DR is the most important reason of irreversible blindness in working-age adults. Many researchers have shown that duration of DM, blood glucose, blood pressure and blood lipids are important risk factors for DR[12]. The duration is the most important factor[13]. To the best of our knowledge, the present study is the first one that is designed to investigate the correlation between smoking severity and DR in newly diagnosed patients with T2DM. The

results suggest that heavy smoking may be an independent risk factor for DR (OR=2.336, p=0.027) in obese newly diagnosed T2DM patients (BMI≥28kg/m^2).

Cigarette smoking is a known risk factor for many diseases, including various kinds of cancer and cardiovascular diseases. Many researchers have reported the negative effects of smoking for DM. A 14-year prospective cohort study in South Korean showed that the risk of DM among men and women who smoked 20 or more cigarettes a day was 1.55 times than non-smokers[7] . A research in the United States and South Korea found that men who smoked before age 20 had significantly higher rates of DM[14]. New quitters had an increased risk of diabetes, but the risk reduced significantly as time went on[15]. A six-year retrospective cohort study showed that quitting smoking would not make people weight gain, but increased the risk of new diabetes in the short term[16]. Cigarette smoking is independently associated with the incidence of type 2 diabetes mellitus (DM). Although the mechanisms involved in the association are not clear yet. Smoking is associated with insulin resistance, inflammation and dyslipidemia[17]. A study showed that adiponectin concentrations might mediate the effects of smoking on diabetes, but leptin and CRP levels did not[18].

Generally, several studies have shown that smoking has an adverse effect on diabetic nephropathy, but the influence of smoking independently with glucose control on retinopathy are unclear. Some studies reported that no association was found between smoking and retinopathy in patients with T2DM[19]. The United Kingdom Prospective Diabetic (UKPD) study which to determine risk factors related to the incidence and progression of diabetic retinopathy followed patients over 6 years from diagnosis of Type II (non-insulin-dependent) diabetes mellitus. The development of retinopathy was associated with glycemia and higher blood pressure, but not smoking [20]. Thus in patients with T2DM, the effect of smoking on diabetic retinopathy is not so clear compared with nephropathy. 20% of newly diagnosed type 2 diabetes mellitus(T2DM) are diagnosed with DR[3]. The explanation of this phenomenon is that the onset of T2DM is usually insidious, and many patients with diabetes have been suffering from diabetes for many years before being diagnosed. Although we cannot completely rule out the relevance between known duration of DM and the prevalence of DR, our study suggests that smoking may be a risk factor for the occurrence of DR in newly diagnosed diabetic patients. Basic research shows that smoke from burning cigarettes contains a large variety of compounds, including nicotine, cadmium, benzapyrene, oxidants and free radicals. These substances cause vasoconstriction, leading to local hypoxia. The early phenomenon of smoke toxicity is vascular endothelial dysfunction. Studies have shown that cigarette smoking impairs nitric oxide-mediated endothelial function via increased generation of superoxide anion, which may increase the risk of diabetic retinopathy in diabetic patients[21]. Jaimes EA et al. found that short exposure (for 30 minutes) of bovine pulmonary arterial endothelial cells, human pulmonary

artery endothelial cells and rat pulmonary artery to cigarette smoke extracts, resulted a large increase in superoxide anion production which was prevented by prior inhibition of NADPH oxidase. So the study reported compounds of cigarette smoke by NADPH oxidase activation induced endothelial superoxide anion production which caused vascular endothelial dysfunction[22], can cause eye tissue ischemia, eye tissue hypoxia, retinal arteriosclerosis and decrease of choroidal blood flow, eventually leading to retinal ischemia. Then DR happens finally. Tyrberg M et al.investigated 794 patients diagnosed with diabetes in 1987-1988 and found that smoking history could increase the risk of diabetic retinopathy during 9 to 17 years after diagnosis[23]. In our investigation, the incidence of DR was 18.0% in non-smokers, 19.8% in light and moderate smokers, and 26.1% in heavy smokers. Higher smoking amount maybe a risk factor of getting DR in newly diagnosed T2DM patients.

In the study, we found that DR was negatively correlated with BMI(OR=0.634,p=0.012).Gray et al. followed up 14,657 diabetic patients for an average of 6.68 years and found that increased risk of DR was associated with higher BMI[24]. It had also been reported that BMI was not correlated or even negatively correlated with DR[25]. The subjects in our study were newly diagnosed type 2 diabetes mellitus. The average age of newly T2DM patients with obesity(BMI≥28kg/m^2) was 50.4±12.4 years, while that of patients without obesity was 55.1± 10.6 years. The former was significantly lower than the latter(t=6.094, p<0.001). As the onset of T2DM is insidious, patients may have a long history before diagnosis. Based on the usual clinical experience and their different diagnostic ages, our study suggests that the diagnostic age of T2DM patients with obesity is closer to the real age of onset. When BMI was ≥28 kg/m^2, we found that the incidence of DR was 12.4% in non-smokers, 12.1% in light and moderate smokers, and 28.2% in heavy smokers(χ^2=11.163,p=0.004). Heavy smoking was significantly associated with DR(OR=2.336, p=0.027).

5. Conclusion

Our study found that heavy smoking was an independent risk factor for the occurrence of DR in newly diagnosed diabetes mellitus patients when BMI was ≥28 kg/m^2. Although our study subjects were with all newly diagnosed patients, it was known that the onset of type 2 diabetes mellitus was relatively insidious, and especially HbA1c levels of all subjects of our study were higher than 9%. So their course of disease may have been last for a period of time. And we can also speculate that DR is associated with smoking status in pre-diabetes. It is of great significance to quit smoking in high-risk population of diabetes mellitus. Therefore, to elucidate the correlation clearly, we need to do a long-term cohort study with large sample to confirm.

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Competing interests None declared.

Patient consent Obtained.

Ethics approval This study was approved by the IRB of Tianjin 4th Central Hospital(2017-SZXL017) and the Chinese Clinical Trial Registry (No. ChiCTR1900020728).

Provenance and peer review Not commissioned; externally peer reviewed.

Contributors Zhaohu Hao wrote the manuscript. Xiao Huang was in charge of fundus examination. Yongzhang Qin was responsible for basic information collection. Hailin Shao was responsible for the statistical analysis of the data. Huanming Li was responsible for auditing the fundus images and reports. Rong Xu was responsible for keeping patients in order. Baocheng Chang proposed the necessity and design of the study. Fengshi Tian was the director of our department and also provided project support for the research.

Data sharing statement Extra data is available by emailing keddyhm@163.com

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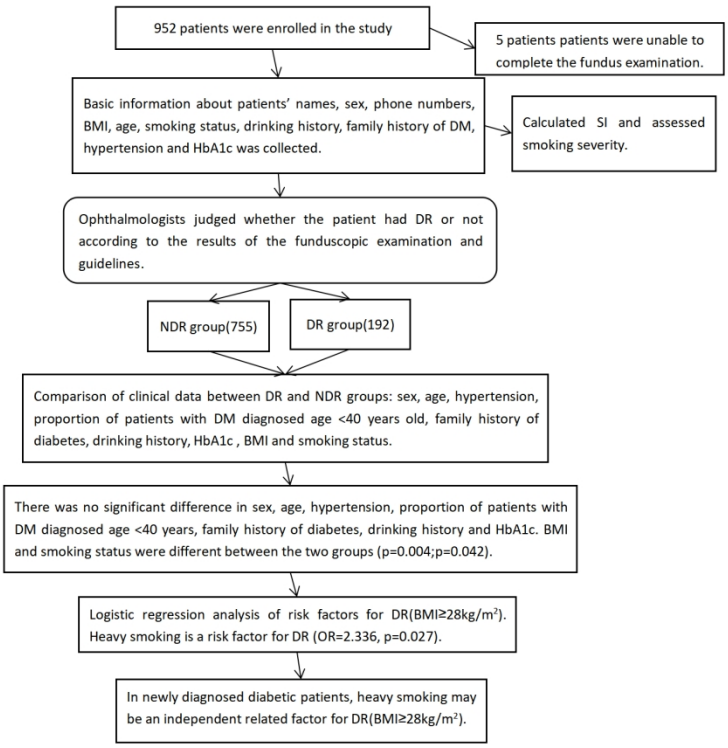
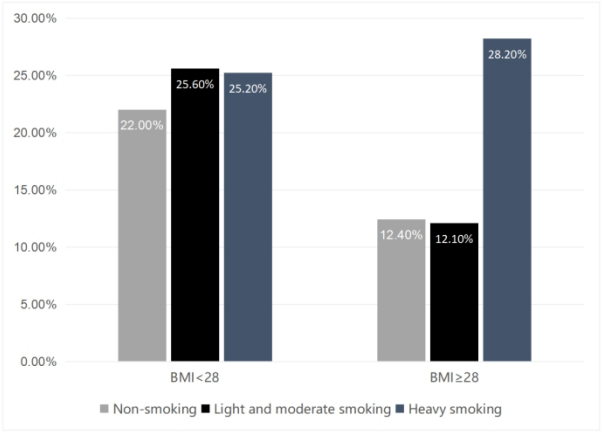
Figure 1 The clinical trial flow chart.

Abbreviations: BMI, body mass index; SI, smoking index; DR, diabetic retinopathy; NDR, non-diabetic retinopathy; DM, diabetes mellitus.

Figure 2 The incidence of DR in patients with different smoking status

Note: There was no difference in DR incidence among patients with different smoking status(BMI<28kg/m^2,χ^2=0.901,p=0.634); When the patients were obese(BMI≥28kg/m^2), the DR detection rate of severe smokers was significantly higher(χ^2=11.163,p=0.004).


952 patients were enrolled in the study
5 patients patients were unable to complete the fundus examination.
Basic information about patients' names, sex, phone numbers, BMI, age, smoking status, drinking history, family history of DM, hypertension and HbA1c was collected.
Calculated SI and assessed smoking severity.
Ophthalmologists judged whether the patient had DR or not according to the results of the funduscopic examination and guidelines.
NDR group(755)
DR group(192)
Comparison of clinical data between DR and NDR groups: sex, age, hypertension, proportion of patients with DM diagnosed age <40 years old, family history of diabetes, drinking history, HbA1c , BMI and smoking status.
There was no significant difference in sex, age, hypertension, proportion of patients with DM diagnosed age <40 years, family history of diabetes, drinking history and HbA1c. BMI and smoking status were different between the two groups (p=0.004;p=0.042).
Logistic regression analysis of risk factors for DR(BMI≥28kg/m²). Heavy smoking is a risk factor for DR (OR=2.336, p=0.027).
In newly diagnosed diabetic patients, heavy smoking may be an independent related factor for DR(BMI≥28kg/m²).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25.20%
12.40%
12.10%
28.20%
BMI<28
BMI≥28
Non-smoking
Light and moderate smoking
Heavy smoking

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Analysis of factors related to diabetic retinopathy in newly diagnosed type 2 diabetic patients: a cross-sectional study

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Analysis of factors related to diabetic retinopathy in newly diagnosed type 2 diabetic patients: a cross-sectional study. (Tables = 4, Figures = 2)

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Abstract

Aim: To investigate the related factors of DR, and explore the correlation between smoking and DR in patients with newly diagnosed type 2 diabetes who visited in the hospital from December 2018 to April 2019.

Design: A single-centre cross-sectional study.

Setting: Single-centre, large hospital.

Participants: Patients with newly diagnosed type 2 diabetes who visited the hospital from December 2018 to April 2019.

Methods: 947 patients were enrolled in the study. They were divided into two groups according to whether they were diagnosed with DR on presentation (diabetic retinopathy group and non diabetic retinopathy group). The smoking index and assessed smoking severity were calculated. Factors such as sex, age, hypertension, proportion of patients with T2DM diagnosed age <40 years old, family history of diabetes, drinking history, HbA1c, BMI and smoking status were compared between two groups, and logistic regression was used to analyzing the relationship between DR and the above factors.

Results There was no statistically significant difference between the two groups in sex, age, hypertension, proportion of patients with DM diagnosed age <40 years old, family history of diabetes, drinking history and HbA1c. Body mass index (BMI) was significantly higher in DR patients (27.7 ± 4.2vs26.7 ± 4.4, p=0.004). Smoking status was also different between the two groups (χ^2=6.350, p=0.042). BMI was shown to be a related factor for DR in newly diagnosed diabetic patients (OR=0.634, p=0.012) by logistic regression analysis. When BMI was ≥28 kg/m^2, heavy smoking was significantly associated with DR (OR=2.336, p=0.027).

Conclusions Heavy smoking was an important related factor for DR in newly diagnosed diabetes mellitus patients when BMI was ≥28 kg/m^2. To elucidate the correlation clearly, we need to do a long-term cohort study with a large sample to confirm this.

Keywords: Smoking index, Type 2 diabetes mellitus, Diabetic retinopathy, newly diagnosed diabetes mellitus.

Strengths and limitations of this study

This study is a representative population-based study with large sample size.

There are few studies on the factors related to newly diagnosed diabetic retinopathy in type 2 diabetes mellitus patients in China.

In this study, we first used a smoking index to assess smoking severity in the study of diabetic retinopathy.

The study is a single-centre cross-sectional study.

Introduction:

Diabetes mellitus (DM) is a chronic metabolic disease characterized by hyperglycemia caused by various etiologies, and the incidence is increasing year by year worldwide. According to the report of the International Diabetes Federation (IDF) in 2017 about 425 million people are affected by DM, and that number is expected to reach 629 million by 2045.[1] China has the highest number of patients with diabetes, and the prevalence has increased from 0.67% in 1980 to 10.4% in 2013.[2] A meta-analysis based epidemiological study conducted from 35 research centers around the world showed that the prevalence of diabetic retinopathy(DR) in the total diabetic population was 35.4%.[3] DR is a highly specific microvascular complication caused by diabetes, and its prevalence is significantly correlated with the course of DM and blood glucose levels.[4] DR is the most important cause of irreversible blindness in working-age adults. Chronic hyperglycemia causes not only retinal vascular diseases, but also damage to retinal neurons, both of which factors lead to vision loss.[5] Twenty percent of newly diagnosed type 2 diabetic (T2DM) patients are diagnosed with DR. The explanation of this phenomenon is that often the onset of T2DM is insidious, and patients may have a long history of untreated hyperglycemia before diagnosis.[3] Are there any other risk factors for DR in these newly diagnosed patients? There is no relevant research report at present.

Smoking is harmful and it's also an important risk factor for cardiovascular, cerebrovascular and, respiratory diseases and malignant tumors.[6] Smoking is one of the most serious public health issues across the world and it is responsible for about 7 million deaths per year worldwide .[6] In a 14-year prospective cohort study, Jee SH found that people smoking more than 20 cigarettes a day had a 1.55-fold higher incidence of diabetes than non-smokers.[7] It is well-known that smoking may cause an increase in proteinuria. Everyone speculates that smoking has a adjunct effect on DR, but there is no direct clinical epidemiological evidence supporting the suggestion that there is a direct link between smoking and DR in newly diagnosed patients.[8] Many diabetic patients had started smoking before they were diagnosed with diabetes. Does smoking contribute to the complications of DR in newly diagnosed patients? Little research has been done on the relationship between smoking and DR in newly diagnosed diabetic patients. The present study investigated the factors related to DR, and explored the correlation between smoking and DR in patients with newly diagnosed type 2 diabetes who visited in the hospital from December 2018 to April 2019.

Research design and methods:

We conducted a single-center cross-sectional study of factors associated with diabetic

retinopathy in newly diagnosed type 2 diabetic patients at Tianjin 4th Central Hospital. The hospital is a high-level comprehensive hospital with 1000 beds, which is a regional medical center in Tianjin. The hospital has a diabetes identification center. New diabetic patients in the region must be identified by the department before they can enjoy more preferential health insurance policies in the clinic. The Metabolic Disease Management Center is responsible for the screening for complications in newly diagnosed diabetes patients, such as urinary protein, fundus examination, blood vessel and nerve conduction. Initially, all patients with high blood glucose were identified and classified by the diabetes identification center. Electronic medical records were generated at this time. Next, the patients entered the metabolic disease management center to complete the screening for diabetic retinopathy. Concurrently, the diabetes nursing team undertook the measurement of height, weight and blood pressure, and gathered information about smoking, drinking and past disease history.

Participants: Study participants were newly diagnosed type 2 diabetic patients who visited the Metabolic Disease Management center of the Tianjin 4th Central Hospital from December 2018 to April 2019. The patients information came from an electronic database of newly diagnosed type 2 diabetics at the hospital's diabetes identification center. All the patients who underwent fundus examination were enrolled in this study. All patient identifiers were removed prior to analysis. This clinical study protocol was approved by the institutional review board of Tianjin 4th Central Hospital, and all steps were conducted in accordance with the principles the World Medical Association Declaration of Helsinki.[9] (Trial registration code: ChiCTR1900020728).Written informed consent was obtained from each patient.

Inclusion criteria:

The study enrolled participants with a baseline of newly diagnosed with T2DM within past six months; patients who could communicate independently and describe their smoking status; none of the smokers had given up smoking before the study; all patients underwent diabetic retinopathy screening in the study.

According to the Chinese guidelines for the prevention and treatment of diabetes, all patients with diabetes in our hospital need to be diagnosed by an oral glucose tolerance test. The diagnostic criteria during execution are as follows:(1)Fasting plasma glucose(FPG)≥7.0mmol/l. Fasting is defined as no caloric intake for at least 8h; Or(2)2-h plasma glucose≥11.1mmol/l during an oral glucose tolerance test(OGTT). The test should be performed as described by the World Health Organization, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in the water. HbA1c test is not standardized in China, so it cannot be used as a

diagnostic standard.[10]

Exclusion criteria:

Exclusion criteria : Type 1 diabetes and other specific types of diabetes; acute complications of diabetes, such as diabetic ketoacidosis or lactic acidosis; hypovolaemia; subjects with diabetic nephropathy complications, rheumatic diseases, autoimmune diseases, pregnancy, malignant tumors, infection, foot ulcer, severe mental illness, thyroid dysfunction, severe hepatic insufficiency or renal insufficiency, severe anemia, heart failure or respiratory insufficiency; cognitive impairment, and communication disorders.

Evaluation of Clinical variables:

Information about patients' names, sex, phone numbers, Body mass index (BMI), age, smoking history, drinking history, family history of DM, hypertension and HbA1c was collected by using a uniform information table. The formula for BMI is weight in kilograms divided by height in meters squared.BMI (≥28kg/m^2) met obesity criteria according to Chinese standards. Venous blood samples were collected by EDTA tubes in fasting state in the morning. The level of HbA1c was determined by affinity chromatography in the hospital standard laboratory (**Tosoh Corporation, Japan**). Smoking status assessment: according to the WHO (1984) smoking survey method, smoking at least one cigarette per day for more than one year is considered as a smoking history. The smoking index is calculated by multiplying the number of cigarettes smoked per day by the number of years the person has smoked. In our study, according to the smoking index, 0 means non-smoking, ≤500 means light and moderate smoking, and > 500 means heavy smoking. of T2DM, subjects were divided into two groups according to the age of diagnosis (≥40 or <40 years old).[11]

Screening for DR was carried out according to EURODIAB guidelines using one 45° field retinograph, centered on the fovea. If DR was suspected, another two 45° retinographs of were taken.[12] Ophthalmologists judged whether the patient had DR or not according to the results of the funduscopic examination and guidelines. In this study, the results were reported as either positive or negative (diabetic retinopathy DR or non-diabetic retinopathy NDR), and the patients were divided into two groups (DR group and NDR group). Non-diabetic retinopathy here includes a normal fundus and other retinopathy that may be caused by other causes (non-diabetic) such as fundus arteriosclerosis.

Statistical analysis:

Descriptive analysis was used to illustrate the basic demographic characteristics and past medical history of the enrolled population. For continuous variables, one sample Kolmogorov-Smirnov Normality test was used to check normality of the distribution of such variables. Once normal distribution and homogeneity of variance were satisfied, values were represented by the mean ±SD and student t-test was used to compare the difference between two groups. For categorical variables, chi-squared test was performed. The occurrence of DR was identified as the dependent variable, and the related factors (sex, hypertension, age at DM diagnosis, family history of diabetes, drinking history, HbA1c, BMI and smoking status) were listed as independent variables. We examined the factors associated with DR using a multivariable logistic regression analysis. BMI could be a possible confounding variable. According to whether BMI ≥ 28kg/m^2, multiple regression analysis was made on the above factors..All the analyses were conducted using SPSS 16.0 software. A significant difference exists if $p<0.05$ (two-sides).

Patient and Public Involvement:

Patients were not involved in setting the research questions or planning the study. Investigators did not know the identities of the study participants. In this study, we accessed patient's electronic data from the health records at our institution. All patient identifiers were removed before the analysis was conducted. There was no direct patient and public involvement.

Results:

The flow chart of the cross-sectional study is shown in Figure 1. Initially, 952 patients entered the study and 5 were excluded because we were unable to see the fundus. Finally, a total of 947 patients were enrolled in the study. 430 patients had a history of smoking. The average smoking commencement age was 27.8±12.2 years (2~60 years). They smoked 21.8± 12.3 cigarettes per day. Baseline characteristics are shown in Table 1.

The comparison of clinical data between DR and NDR groups is shown in Table 2. There was no statistically significant difference between the two groups in sex, age, hypertension, proportion of patients with DM diagnosed age <40 years old, family history of diabetes, drinking history and HbA1c. BMI was significantly higher in DR patients (27.7±4.2 vs 26.7±4.4, p=0.004). We found that smoking status was different between the two groups (χ^2=6.350, p=0.042).

The occurrence of DR was identified as the dependent variable, and the related risk factors were listed as independent variables. Both of the variables were progressed through a multivariate logistic regression analysis in Table 3. Patients with BMI≥28kg/m^2 were less likely to have DR in newly diagnosed diabetic patients (OR=0.634, p=0.012).

The average age of newly T2DM patients with obesity was 50.4±12.4 years, while that of patients without obesity was 55.1±10.6 years. The former was significantly lower than the latter (t=6.094, p<0.001). Because the onset of T2DM is insidious, patients may have a long history before diagnosis.[3] BMI could be a possible confounding variable. We divided the participants into two groups according to whether BMI was ≥ 28 kg/m^2. We compared the incidence of DR in patients with different smoking status (Figure 2), and used logistic regression analysis to analysis the related factors of DR (Table 4). When BMI was ≥28 kg/m^2, we found that heavy smoking was significantly associated with DR (OR=2.336, p=0.027).

Table 1 Baseline characteristics of participants with newly diagnosed T2DM.

Characteristics of participants	Comment	Statistical description
Sex（n，%）	Male	566（59.78%）
Age（years）		53.2±11.6
Family history of DM（n，%）		580（61.2%）
Age at diagnosis of DM（n，%）	< 40 years old	147（15.5%）
HbA1c（%）		9.53±2.13
BMI（kg/m^2）		27.5±4.3
Hypertension（n，%）		498（52.6%）
Drinking history (n,%)		195（20.6%）
Smoking status（n，%）	Non-smoking	517（54.6%）
	Light and moderate smoking	212（22.4%）
	Heavy smoking	218（23.0%）
The number of years a person has smoked（years）		27.8±12.2

DR（n，%）	192（20.3%）
PDR（n，%）	12（1.3%）

Abbreviations： DR, diabetic retinopathy; PDR, proliferative diabetic retinopathy.

Table 2 Comparison of clinical data between DR and NDR groups

Items		NDR（n=755）	DR（n=192）	t/χ^2	P value
Sex	Male	447（79.0%）	119（21.0%）	0.490[a]	0.510
	Female	308（80.8%）	73（19.2%）		
Age	years	53.3±11.7	52.9±11.1	0.350[b]	0.726
Family history of DM	no	288（78.5%）	79（21.5%）	0.580[a]	0.456
	yes	467（80.5%）	113（19.5%）		
Drinking history	no	608（80.9%）	144（19.1%）	2.863[a]	0.109
	yes	147（75.4%）	48（24.6%）		
Hypertension[c]	no	349（81.2%）	81（18.8%）	1.457[a]	0.265
	yes	392（78.7%）	106（21.3%）		
DM diagnosed age <40	no	123（83.7%）	24（16.3%）	1.678[a]	0.220
	yes	632（79.0%）	168（21.0%）		
HbA1c	%	9.51±2.13	9.60±2.12	-0.546[b]	0.585
BMI	kg/m^2	27.7±4.2	26.7±4.4	2.921[b]	0.004
Smoking history	no	424(82.0%)	93(18.0%)	3.682[b]	0.062
	yes	331(77.0%)	99(23.0%)		
Smoking status	Non-	424（82.0%）	93（18.0%）	6.350[a]	0.042

smoking		
Light and moderate smoking	170（80.2%）	42（19.8%）
Heavy smoking	161（73.9%）	57（26.1%）

Note: [a] chi-square test , [b] student t test and [c]19 cases of patients with missing data. There was no statistically significant difference if p >0.05.

Table 3 Logistic regression analysis of related factors for DR

Independent variables	B	SE	Wald χ^2	P value	OR	95%CI
Sex	0.085	0.224	0.144	0.705	1.089	0.702-1.688
Hypertension	0.171	0.172	0.993	0.319	1.187	0.847-1.663
Drinking history	0.178	0.219	0.659	0.417	1.195	0.778-1.835
HbA1c	0.075	0.168	0.197	0.657	1.077	0.775-1.498
BMI	-0.456	0.181	6.334	0.012	0.634	0.445-0.904
Family history of DM	-0.125	0.170	0.547	0.460	0.882	0.633-1.230
DM diagnosed age <40 years	0.011	0.263	0.002	0.966	1.011	0.604-1.692
Smoking status			3.479	0.176		
Light and moderate smoking	0.087	0.249	0.121	0.728	1.091	0.669-1.777
Heavy smoking	0.427	0.244	3.056	0.080	1.533	0.950-2.474
Constant	-1.579	0.631	6.259	0.012	0.206	

Note: assignment instructions as follows---Sex (male 1, female 2); BMI(<28 0, ≥28 1); HbA1c(≤9% 0, >9 1); Hypertension、drinking history、family history of DM and DM diagnosed age <40 years no 0, yes 1; Smoking status non-smoking 0, light and moderate smoking 1, heavy smoking 2, non-smoking as the reference.

Table 4 Logistic regression analysis of related factors for DR (BMI<28kg/m^2, ≥28kg/m^2)

	Independent variables	B	SE	Wald χ^2	P value	OR	95%CI
BMI<28 kg/m^2	Sex	-0.167	0.283	0.347	0.556	1.182	0.678-2.060
	Hypertension	0.070	0.212	0.107	0.743	1.072	0.707-1.625
	Drinking history	0.059	0.275	0.046	0.830	1.061	0.619-1.817
	HbA1c	-0.025	0.205	0.015	0.902	0.975	0.653-1.457
	Family history of DM	-0.368	0.204	3.247	0.072	0.692	0.464-1.033
	DM diagnosed age <40 years	0.099	0.380	0.068	0.794	1.104	0.525-2.324
	Smoking status			1.037	0.595		
	Light and moderate smoking	0.278	0.318	0.766	0.382	1.321	0.720-2.506
	Heavy smoking	0.295	0.318	0.858	0.354	1.343	0.950-2.474
BMI≥28 kg/m^2	Sex	-0.009	0.382	0.001	0.980	0.991	0.468-2.096
	Hypertension	0.345	0.313	1.220	0.269	1.412	0.765-2.607
	Drinking history	0.465	0.377	1.526	0.217	1.592	0.761-3.331
	HbA1c	0.335	0.304	1.213	0.271	1.398	0.770-2.536
	Family history of DM	0.449	0.325	1.905	0.167	1.566	0.828-2.962
	DM diagnosed age <40 years	0.046	0.376	0.015	0.902	1.047	0.501-2.187
	Smoking status			7.156	0.028		
	Light and moderate smoking	-0.173	0.422	0.169	0.681	0.841	0.368-1.922
	Heavy smoking	0.848	0.384	4.879	0.027	2.336	1.100-4.960

Discussion:

Overall results of this survey:

In the study, among the newly diagnosed T2DM patients 20.3% presented with DR. After

analyzing the related factors of DR in newly diagnosed T2DM patients, our study found that obese patients may have a lower incidence of DR (OR=0.634, p=0.012). Many researchers have shown that duration of DM, blood glucose, blood pressure and blood lipids are important risk factors for DR.[13-15] The duration is the most important factor.[16] But in our study, we found that DR was only related to body mass index and smoking in newly diagnosed T2DM patients. To the best of our knowledge, the present study is the first one that is designed to investigate the correlation between smoking level and DR in newly diagnosed patients with T2DM. The results of the study suggest that heavy smoking was associated with DR in obese (BMI⩾28kg/m2) newly diagnosed T2DM patients (OR=2.336, p=0.027), while heavy smoking was not associated with DR in non-obese (BMI<28kg/m2) subjects (OR=1.343,p=0.354). .

The relationship between BMI and DR

In the study, we found that DR was negatively correlated with BMI. This is not consistent with other research results. Gray et al. followed up 14,657 diabetic patients for an average of 6.68 years and found that increased risk of DR was associated with higher BMI.[17] It had also been reported that BMI was not correlated or even negatively correlated with DR.[18] The subjects in our study had newly diagnosed type 2 diabetes mellitus. The average age of newly T2DM patients with obesity (BMI≥28kg/m^2) was 50.4±12.4 years, while that of patients without obesity was 55.1±10.6 years. The former was significantly lower than the latter (t=6.094, p<0.001). As the onset of T2DM is insidious, patients may have had a long history before diagnosis.[3] Based on the usual clinical experience and their different diagnostic ages, our study suggests that the diagnostic age of T2DM patients with obesity is closer to the real age of onset. Perhaps because non-obese patients may be confused with more complex diabetic course factors, we did not find any factors in the analysis of the related factors of DR. In conclusion, we don't think that high BMI is a protective factor for DR. It is very likely that there are confounding factors, such as an unobserved course of T2DM.

The relationship between smoking and DR:

Cigarette smoking is a well-known risk factor for many chronic diseases, including cardiovascular diseases and various malignancies. Smoking is associated with higher insulin resistance, chronic inflammation, and dyslipidemia. Smoking is strongly linked with both increased incidence as well as severity of diabetes. Smoking has also been shown to be associated with a higher risk of diabetes complications especially macrovascular complications. Smoking cessation is one of the important targets for diabetes control and the prevention of chronic diabetes complications.[19] When BMI was ≥28 kg/m^2, we found that the incidence of DR was 12.4% in non-smokers, 12.1% in light and moderate smokers, and 28.2% in heavy smokers (χ^2=11.163,p=0.004).Many researchers have reported the negative effects of smoking on DM.[7]

[20-21] A 14-year prospective cohort study in South Korea showed that the risk of DM among men and women who smoked 20 or more cigarettes a day was 1.55 times higher than in non-smokers.[7] Cigarette smoking is independently associated with the incidence of type 2 diabetes mellitus.[22] Although the mechanisms involved in the association are not clear yet. Smoking is associated with insulin resistance, inflammation and dyslipidemia.[23-24] Some studies have reported that no association was found between smoking and retinopathy in patients with T2DM.[25] The United Kingdom Prospective Diabetic (UKPD) study which followed patients over 6 years from diagnosis of Type II (non-insulin-dependent) diabetes mellitus. in order to determine risk factors related to the incidence and progression of diabetic retinopathy found that the development of retinopathy was associated with glycemia and higher blood pressure, but not smoking.[26]

Studies have shown that cigarette smoking impairs nitric oxide-mediated endothelial function via increased generation of superoxide anions, which may increase the risk of diabetic retinopathy in diabetic patients and could cause eye tissue ischemia, eye tissue hypoxia, retinal arteriosclerosis and decrease of choroidal blood flow, eventually leading to retinal ischemia.[27-28] This finally leads to DR . Based on the usual clinical experience and their different diagnostic ages, our study suggests that the diagnostic age of T2DM patients with obesity is closer to the real age of onset. When BMI was ≥28 kg/m^2, present study shows that the incidence of DR was 28.2% in heavy smokers. Heavy smoking was significantly associated with DR. Arterial stiffness has been associated with diabetic retinopathy. A Logistic regression model was used to evaluate the relationship between diabetic retinopathy and pulse wave velocity and enhancement index, and this concluded that central arterial stiffness was associated with the presence and severity of diabetic retinopathy in type 2 diabetes mellitus patients, suggesting its etiologic is implicated in diabetic retinopathy.[29] Smoking may aggravate retinopathy damage by increasing arteriosclerosis. Diabetic retinopathy remains the leading cause of acquired blindness in working-age adults. While the cutting-edge research in the field has identified many molecular, functional, and structural abnormalities, the exact molecular mechanism of this devastating disease remains obscure.[30] A diabetic environment drives the dysfunction of the power generator of the cell and disturbs the homeostasis of the mitochondrial dynamic. Mitochondria appear to have a significant role in the development of diabetic retinopathy, and unraveling the mechanism responsible for their damage as well as the role of epigenetic modifications in mitochondrial homeostasis should identify novel therapeutic targets.[30] Further research is needed to determine whether smoking affects mitochondrial DNA function.

In the present study, DR was found in 20.3% of newly diagnosed type 2 diabetic patients. This was consistent with other findings.[3] The traditional explanation of this phenomenon is that the onset of T2DM is usually insidious, and many patients with diabetes have been suffering

from diabetes for many years before diagnosis.[3] In our investigation, the incidence of DR was 18.0% in non-smokers, 19.8% in light and moderate smokers, and 26.1% in heavy smokers. Although we cannot completely rule out the relevance between known duration of DM and the prevalence of DR, our study suggests that smoking may be an important related factor for the occurrence of DR in newly diagnosed diabetic patients. Tyrberg M et al. investigated 794 patients diagnosed with diabetes in 1987-1988 and found that smoking history could increase the risk of diabetic retinopathy during the 9 to 17 years after diagnosis.[31] Because smoking behavior occurs before the diagnosis of T2DM in this study, heavy smoking is very likely to be a risk factor for DR. Prospective research is needed to confirm this view.

Limitations of the study

The study is a single-center cross-sectional study. The results only show the correlation between smoking and diabetic retinopathy. Prospective cohort studies are needed to confirm whether there is a causal relationship between smoking and DR in newly diagnosed T2DM patients. Because of the large number of newly diagnosed type 2 diabetes patients, we have set relatively wide exclusion criteria for the population, which may affect external validity. Although our department is responsible for the first diagnosis and identification of diabetes in Tianjin, it is still unable to achieve a timely diagnosis for all those patients who newly develop diabetes. Because the patients are all from Tianjin, China, the conclusion has got regional limitations.

Conclusion

Our study found that heavy smoking was an important related factor for the occurrence of DR in newly diagnosed diabetes mellitus patients when BMI was ≥28 kg/m^2. Although our study subjects were with all newly diagnosed patients, it was known that the onset of type 2 diabetes mellitus had been relatively insidious; especially HbA1c levels of all subjects of our study were higher than 9%. So the course of their disease may have lasted for a prolonged period of time. And we can also speculate that DR is associated with smoking status in pre-diabetes. It is of great importance for people in populations at high-risk of diabetes mellitus to quit smoking. Therefore, to elucidate the correlation clearly, we need to do a long-term cohort study with large sample to confirm this.

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Contributors: Zhaohu Hao wrote the manuscript. Xiao Huang was in charge of fundus examination. Yongzhang Qin was responsible for basic information collection. Hailin Shao was responsible for the statistical analysis of the data. Huanming Li was responsible for auditing the fundus images and reports. Rong Xu was responsible for keeping patients in order. Baocheng Chang proposed the necessity and design of the study. Fengshi Tian was the director of our department and also provided project support for the research.

Data sharing statement: Data is available upon reasonable request.

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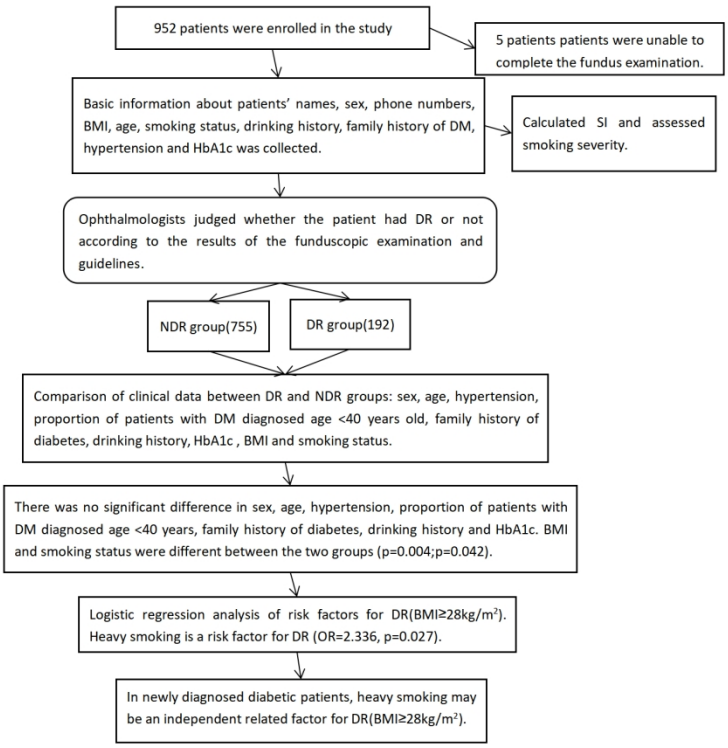
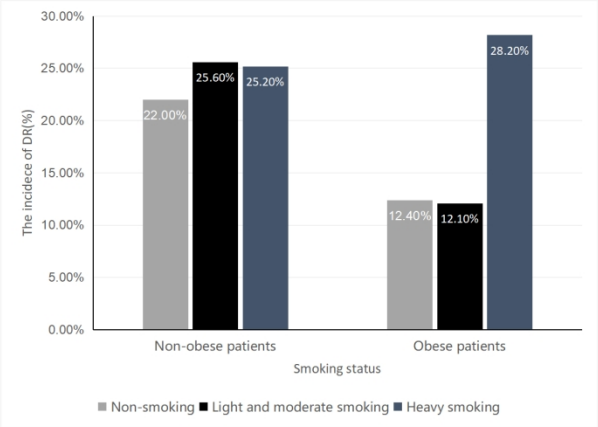
Figure 1 Flow chart of the study.

Abbreviations: BMI, body mass index; SI, smoking index; DR, diabetic retinopathy; NDR, non-diabetic retinopathy; DM, diabetes mellitus.

Figure 2 The incidence of DR in patients with different smoking status

Note: There was no difference in DR incidence among patients with different smoking status(BMI<28kg/m^2,χ^2=0.901,p=0.634); When the patients were obese(BMI≥28kg/m^2), the DR detection rate of severe smokers was significantly higher(χ^2=11.163,p=0.004).


952 patients were enrolled in the study
5 patients patients were unable to complete the fundus examination.
Basic information about patients' names, sex, phone numbers, BMI, age, smoking status, drinking history, family history of DM, hypertension and HbA1c was collected.
Calculated SI and assessed smoking severity.
Ophthalmologists judged whether the patient had DR or not according to the results of the funduscopic examination and guidelines.
NDR group(755)
DR group(192)
Comparison of clinical data between DR and NDR groups: sex, age, hypertension, proportion of patients with DM diagnosed age <40 years old, family history of diabetes, drinking history, HbA1c , BMI and smoking status.
There was no significant difference in sex, age, hypertension, proportion of patients with DM diagnosed age <40 years, family history of diabetes, drinking history and HbA1c. BMI and smoking status were different between the two groups (p=0.004;p=0.042).
Logistic regression analysis of risk factors for DR(BMI≥28kg/m²). Heavy smoking is a risk factor for DR (OR=2.336, p=0.027).
In newly diagnosed diabetic patients, heavy smoking may be an independent related factor for DR(BMI≥28kg/m²).



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209x296mm (192 x 192 DPI)

STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation	Page Number
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	1-2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	2
Objectives	3	State specific objectives, including any prespecified hypotheses	2-3
Methods			
Study design	4	Present key elements of study design early in the paper	3
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	3-4
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	3-4
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	4
Bias	9	Describe any efforts to address potential sources of bias	none
Study size	10	Explain how the study size was arrived at	none
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	4
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	4
		(*b*) Describe any methods used to examine subgroups and interactions	4
		(*c*) Explain how missing data were addressed	none
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	4
		(*e*) Describe any sensitivity analyses	4
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	5
		(b) Give reasons for non-participation at each stage	none
		(c) Consider use of a flow diagram	5
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	5-6
		(b) Indicate number of participants with missing data for each variable of interest	5
Outcome data	15*	Report numbers of outcome events or summary measures	5-6
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear	5-6

		which confounders were adjusted for and why they were included	
		(*b*) Report category boundaries when continuous variables were categorized	6
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	none
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	none
Discussion			
Key results	18	Summarise key results with reference to study objectives	7-9
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	9
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	9
Generalisability	21	Discuss the generalisability (external validity) of the study results	none
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	10

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Analysis of factors related to diabetic retinopathy in newly diagnosed type 2 diabetic patients: a cross-sectional study.

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Keywords:	Smoking index, Diabetic retinopathy < DIABETES & ENDOCRINOLOGY, Type 2 diabetes mellitus, newly diagnosed diabetes mellitus

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Analysis of factors related to diabetic retinopathy in newly diagnosed type 2 diabetic patients: a cross-sectional study. (Tables = 6)

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Abstract

Aim: To investigate the related factors of diabetic retinopathy(DR), and explore the correlation between smoking and DR in patients with newly diagnosed type 2 diabetes.

Design: A single-centre cross-sectional study.

Setting: Tianjin 4th Central Hospital.

Participants: Patients with newly diagnosed type 2 diabetes who visited the outpatient department of the hospital from December 2018 to April 2019.

Methods: 947 patients were enrolled in the study. They were divided into two groups according to whether they were diagnosed with DR(diabetic retinopathy group,DR group and non diabetic retinopathy group,NDR group). The smoking index(SI) was calculated to assess smoking status. Factors such as sex, age, hypertension, T2DM diagnosed age, family history of diabetes, drinking history, HbA1c,body mass index(BMI) and smoking status were compared between two groups, and logistic regression was used to analyzing the relationship between DR and the above factors.

Results There was no statistically significant difference between the two groups in sex, age, hypertension, DM diagnosed age, family history of diabetes, drinking history and HbA1c. BMI was significantly higher in DR patients (27.7±4.2vs26.7±4.4, p=0.004). Smoking status was also different between the two groups (χ^2=6.350, p=0.042). BMI was shown to be a related factor for DR in newly diagnosed diabetic patients (OR=0.587, p=0.004). When BMI was ≥28 kg/m^2, heavy smoking was significantly associated with DR(OR=2.219, p=0.049),and there was a negative correlation between DR and the age of diagnosis of diabetes≥60 years(OR=0.289, p=0.009).

Conclusions Heavy smoking was an important related factor for DR in newly diagnosed diabetes mellitus patients when BMI was ≥28 kg/m^2. Delaying the age of diabetes might prevent the occurrence of DR. To elucidate the correlation,long-term cohort studys with a large sample were needed.

Keywords: Smoking index, Type 2 diabetes mellitus, Diabetic retinopathy, Newly diagnosed diabetes mellitus.

Strengths and limitations of this study

This study is a representative population-based study.

There are few studies on the factors related to newly diagnosed diabetic retinopathy in type 2 diabetes mellitus patients in China.

In this study, we firstly used smoking index to assess smoking severity in the study of diabetic retinopathy.

The study is a single-centre cross-sectional study.

Introduction:

Diabetes mellitus (DM) is a chronic metabolic disease characterized by hyperglycemia caused by various etiologies, and the incidence is increasing year by year worldwide. According to the report of the International Diabetes Federation (IDF) in 2017 about 425 million people are affected by DM, and that number is expected to reach 629 million by 2045.[1] China has the highest number of DM patients, and the prevalence has increased from 0.67% in 1980 to 10.4% in 2013.[2] A meta-analysis based epidemiological study conducted from 35 research centers around the world showed that the prevalence of diabetic retinopathy(DR) in the total diabetic population was 35.4%.[3] DR is a highly specific microvascular complication caused by diabetes, and its prevalence is significantly correlated with the course of DM and blood glucose levels.[4] DR is the most important cause of irreversible blindness in working-age adults.[4] Chronic hyperglycemia causes not only retinal vascular diseases, but also damage to retinal neurons, both of which factors lead to vision loss.[5] Twenty percent of newly diagnosed type 2 diabetic (T2DM) patients are diagnosed with DR.[3] The explanation of this phenomenon is that often the onset of T2DM is insidious, and patients may have a long history of untreated hyperglycemia before diagnosis.[3] Are there any other risk factors for DR in these newly diagnosed patients? There is no relevant research report at present.

Smoking is harmful and it's also an important risk factor for cardiovascular, cerebrovascular and, respiratory diseases and malignant tumors.[6] Smoking is one of the most serious public health issues across the world and it is responsible for about 7 million deaths per year worldwide .[6] In a 14-year prospective cohort study, Jee SH found that people smoking more than 20 cigarettes a day had a 1.55-fold higher incidence of diabetes than non-smokers.[7] It is well-known that smoking may cause an increase in proteinuria.[8] However, many studies show that smoking has no significant correlation with DR.[9] Even some studies in China have shown that smoking is a protective factor for DR.[10] Many diabetic patients had started smoking before they were diagnosed with diabetes. Does smoking contribute to the complications of DR in newly diagnosed patients? Little research has been done on the relationship between smoking and DR in newly diagnosed diabetic patients. The present study investigated the factors related to DR, and explored the correlation between smoking and DR in patients with newly diagnosed type 2 diabetes who visited the outpatient department of the hospital from December 2018 to April 2019.

Research design and methods:

The present study was a single-center cross-sectional study of factors associated with diabetic retinopathy in newly diagnosed type 2 diabetic patients in Tianjin 4th Central Hospital. The hospital was a regional medical center in Tianjin. The hospital had a diabetes identification

center. The patients in the region should be identified by the department before they could enjoy more preferential health insurance policies in the clinic. The Metabolic Disease Management Center(MMC) was responsible for the screening for complications in newly diagnosed diabetes patients, such as urinary protein, fundus examination, peripheral vascular and neuropathy. The diabetes nursing team undertook the measurement of height, weight and blood pressure, and gathered information about smoking, drinking and past disease history. Electronic medical records were generated at the same time.

Participants: Study participants were newly diagnosed type 2 diabetic patients who visited MMC from December 2018 to April 2019. The patients information came from an electronic database of the hospital's diabetes identification center. All the patients who underwent fundus examination were enrolled in this study. All patient identifiers were removed prior to analysis. This clinical study protocol was approved by the institutional review board of Tianjin 4th Central Hospital, and all steps were conducted in accordance with the principles the World Medical Association Declaration of Helsinki.[11] (Trial registration code: ChiCTR1900020728).Written informed consent was obtained from each patient.

Inclusion criteria:

The study enrolled participants with a baseline of newly diagnosed with T2DM within past six months; patients who could communicate independently and describe their smoking status; none of the smokers had given up smoking before the study; all patients underwent diabetic retinopathy screening in the study.

According to the Chinese guidelines for the prevention and treatment of diabetes, all patients with diabetes in our hospital need to be diagnosed by an oral glucose tolerance test. The diagnostic criteria during execution were as follows:(1)Fasting plasma glucose(FPG)≥7.0mmol/l. Fasting was defined as no caloric intake for at least 8h; Or(2)2-h plasma glucose≥11.1mmol/l during an oral glucose tolerance test(OGTT). The test should be performed as described by the World Health Organization, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in the water. HbA1c test was not standardized in China, so it couldn't be used as a diagnostic standard.[12]

Exclusion criteria:

Exclusion criteria : Type 1 diabetes and other specific types of diabetes; acute complications of diabetes, such as diabetic ketoacidosis or lactic acidosis; hypovolaemia; subjects with diabetic

nephropathy complications, rheumatic diseases, autoimmune diseases, pregnancy, malignant tumors, infection, foot ulcer, severe mental illness, thyroid dysfunction, severe hepatic insufficiency or renal insufficiency, severe anemia, heart failure or respiratory insufficiency; cognitive impairment, and communication disorders.

Evaluation of Clinical variables:

Information about patients' names, sex, phone numbers, BMI, age, smoking history, drinking history, family history of DM, hypertension and HbA1c was collected by using a uniform information table. The formula for BMI was weight in kilograms divided by square of height in meters.BMI (≥28kg/m^2) met obesity criteria according to Chinese standards.[13] Venous blood samples were collected by EDTA tubes in fasting state in the morning. The level of HbA1c was determined by affinity chromatography in the hospital standard laboratory (**Tosoh Corporation, Japan**). Smoking status assessment: According to the WHO (1997) smoking survey method, smoking at least one cigarette per day for more than six months was considered as a smoking history. [14] The smoking index(SI) was calculated by multiplying the number of cigarettes smoked per day by the number of years the person has smoked. In our study, according to SI, 0 meant non-smoking, ≤500 meant light and moderate smoking, and > 500 meant heavy smoking.

Screening for DR was carried out according to EURODIAB guidelines using one 45° field retinograph, centered on the fovea. If DR was suspected, another two 45° retinographs of were taken.[15] Ophthalmologists judged whether the patient had DR or not according to the results of the funduscopic examination and guidelines. In the study, the results were reported as either positive or negative (diabetic retinopathy DR or non-diabetic retinopathy NDR), and the patients were divided into two groups (DR group and NDR group). Non-diabetic retinopathy here includes a normal fundus and other retinopathy that may be caused by other causes (non-diabetic) such as fundus arteriosclerosis.

Statistical analysis:

Descriptive analysis was used to illustrate the basic demographic characteristics. For continuous variables, one sample Kolmogorov-Smirnov Normality test was used to check normality of the distribution of such variables. Once normal distribution and homogeneity of variance were satisfied, values were represented by the $\bar{x} \pm SD$ and student t-test was used. For categorical variables, χ^2 test was performed. The occurrence of DR was identified as the dependent variable, and the related factors (sex, hypertension, age at DM diagnosis, family history of diabetes,

drinking history, HbA1c, BMI and smoking status) were listed as independent variables. To analyse the factors associated with DR and adjust for potential confounding effects,we examined the factors associated with DR using multivariable logistic regression analysis.All the analyses were conducted using SPSS 16.0 software with a $p<0.05$ (two-sides) indicating statistical significance.

Patient and Public Involvement:

Patients were not involved in setting the research questions or planning the study. Investigators did not know the identities of the study participants. In this study, the electronic data was gained from the health records at the institution. All patient identifiers were removed before the analysis was conducted. There was no direct patient and public involvement.

Results:

Characteristics of the study population

In this survey,952 patients entered the study Initially and 5 were excluded because their fundus could not be seen clearly. Finally, a total of 947 patients were enrolled in the study. Their average age was 53.2±11.6 years. The average of HbA1c was 9.53±2.13%. BMI of the patients was 27.5±4.3kg/m^2. 430 patients had a history of smoking. The average smoking age was 27.8 ±12.2 years (2~60 years). They smoked 21.8±12.3 cigarettes per day. Baseline characteristics were shown in Table 1.

Comparison of clinical data between DR and NDR groups

755 patients(79.7%) were in NDR group, and 192 patients were in DR group. Among patients in DR group, there were 12 patients with PDR. The comparison of clinical data between DR and NDR groups was shown in Table 2. There was no statistically significant difference between the two groups in sex, age, hypertension, family history of diabetes, drinking history and HbA1c. BMI was significantly higher in NDR patients (27.7±4.2 vs 26.7±4.4, p=0.004). Smoking status was different between the two groups (χ^2=6.350, p=0.042).

The occurrence of DR was identified as the dependent variable, and the related risk factors

were listed as independent variables. Both of the variables were progressed through multivariate logistic regression analysis in Table 3. Patients with BMI≥28kg/m^2 were less likely to have DR in newly diagnosed diabetic patients (OR=0.587, p=0.004). The age classification of DM diagnosis and smoking status were also statistically significant in regression analysis.

Did obesity have protective effect on DR?

The study compared the age, HbA1c, smoking and drinking history, family history of DM and complications between the obese and non obese patients in Table 4(BMI≥28kg/m^2 vs BMI<28kg/m^2). There was no statistically significant difference between the two groups in sex, family history of DM, drinking history, smoking history and HbA1c. The age of obese diabetic patients was significantly lower than that of non obese patients(50.4±12.4 vs 55.2±10.5, t=-6.184, p<0.001).Because the onset of T2DM was insidious, patients might have a long history before diagnosis.[3] BMI could be a possible confounding variable.

After adjustment for BMI and age, DR related factors were further analyzed.Firstly, the participants were divided into two groups according to whether BMI was ≥ 28 kg/m^2. The incidence of DR in patients with different smoking status was compared(Table 5).The incidence of DR in heavy smoking patients was significantly higher in obese patients. There was no significant difference in the incidence of DR with different smoking status among patients over 60 years old(Table 5). Then after adjustment for age, multiple logistic regression analysis was used to analysis the related factors of DR(Table 6). When BMI was ≥28 kg/m^2, heavy smoking was significantly associated with DR (OR=2.219, p=0.049),and there was a negative correlation between DR and the age of diagnosis of diabetes≥60 years(OR=0.289, p=0.009).

Table 1 Baseline characteristics of 947 participants with newly diagnosed T2DM.

Items	n	%
Sex		
male	566	59.8
female	381	40.2
With family history of DM	580	61.2
Age		
< 50 years old	309	32.5
≥50,< 60 years old	318	33.4

≥60 years old	325	34.1
With hypertension	498	52.6
With drinking history	195	20.6
Smoking status		
Non-smoking	517	54.6
Light and moderate smoking	212	22.4
Heavy smoking	218	23.0

Abbreviations：DM, Diabetes mellitus.

Table 2 Comparison of clinical data between DR and NDR groups

Items		NDR (n=755)	DR (n=192)	t/χ^2	P value
Sex	Male	447(79.0%)	119(21.0%)	0.490[a]	0.510
	Female	308(80.8%)	73(19.2%)		
Age	years	53.3±11.7	52.9±11.1	0.350[b]	0.726
Family history of DM	no	288(78.5%)	79(21.5%)	0.580[a]	0.456
	yes	467(80.5%)	113(19.5%)		
Drinking history	no	608(80.9%)	144(19.1%)	2.863[a]	0.109
	yes	147(75.4%)	48(24.6%)		
Hypertension[c]	no	349(81.2%)	81(18.8%)	1.457[a]	0.265
	yes	392(78.7%)	106(21.3%)		
Age of DM diagnosis	< 50 years	247(79.9%)	62（20.1%)	2.382[a]	0.304
	≥50,< 60 years	243(77.1%)	72(22.9%)		

	≥60 years	265(82.0%)	58(18.0%)		
HbA1c	%	9.51±2.13	9.60±2.12	-0.546[b]	0.585
BMI	kg/m^2	27.7±4.2	26.7±4.4	2.921[b]	0.004
Smoking history	no	424(82.0%)	93(18.0%)	3.682[b]	0.062
	yes	331(77.0%)	99(23.0%)		
Smoking status	Non-smoking	424(82.0%)	93(18.0%)	6.350[a]	0.042
	Light and moderate smoking	170(80.2%)	42(19.8%)		
	Heavy smoking	161(73.9%)	57(26.1%)		

Note: [a] chi-square test , [b] student t test and [c]19 cases of patients with missing data. DM, Diabetes mellitus. There was no statistically significant difference if p >0.05.

Table 3 Logistic regression analysis of related factors for DR

Factors	B	SE	Wald χ^2	P value	OR	95%CI
Sex	0.171	0.220	0.607	0.436	1.187	0.771-1.826
Hypertension	0.257	0.174	2.180	0.140	1.293	0.919-1.818
Drinking history	0.137	0.221	0.381	0.537	1.146	0.743-1.769
HbA1c	0.007	0.038	0.037	0.847	1.007	0.936-1.085
BMI	-0.532	0.183	8.497	0.004	0.587	0.410-0.840
Family history of DM	-0.166	0.173	0.924	0.336	0.847	0.604-1.188
Age of DM diagnosis	-0.248	0.111	4.970	0.026	0.780	0.628-0.970
Smoking status	-0.280	0.125	5.017	0.025	0.756	0.591-0.966
Constant	-0.440	0.582	0.570	0.450	0.644	

Note: assignment instructions as follows---Sex (male 1, female 2); BMI(<28 0, ≥28 1); HbA1c(≤9% 0, >9 1); Hypertension 、 drinking history and family history of DM no 0, yes 1;Age of DM

diagnosis: < 50 years 1, ≥50,< 60 years 2, ≥60 years 3; Smoking status non-smoking 0, light and moderate smoking 1, heavy smoking 2.

Table 4 Comparison of clinical data between patients(BMI≥28kg/m^2 vs BMI<28kg/m^2)

Items		BMI≥28kg/m^2 (n=379)	BMI<28kg/m^2 (n=573)	t/χ^2	P value
Sex(n,%)	Male	230(60.7%)	338(59.0%)	0.273[a]	0.637
	Female	149(39.3.8%)	235(41.0%)		
Age	years	50.4±12.4	55.2±10.5	-6.184[b]	<0.001
Family history of DM	no	141(37.2%)	228(39.8%)	0.643[a]	0.455
(n,%)	yes	238(62.8%)	345(60.2%)		
Drinking history(n,%)	no	305(80.5%)	452(78.9%)	0.567[a]	0.305
	yes	74(19.5%)	121(21.1%)		
Hypertension[c](n,%)	no	148(40.1%)	284(50.4%)	9.419[a]	0.002
	yes	221(59.9%)	280(49.6%)		
HbA1c	%	9.38±2.10	9.63±2.30	-1.744[b]	0.082
Smoking history(n,%)	no	217(57.3%)	303(52.9%)	1.763[b]	0.206
	yes	162(42.7%)	270(47.1%)		

Note: [a] chi-square test , [b] student t test and [c]19 cases of patients with missing data. There was no statistically significant difference if p >0.05.

Table 5 The incidence of DR in patients with different smoking status

Items	Non-smoking (n,%)	Light and moderate smoking(n,%)	Heavy smoking (n,%)	χ^2	P value

BMI(kg/m^2)	≥28	27(12.4%)	11(12.1%)	20(28.2%)	11.163	0.004
	<28	66(22.0%)	31(25.6%)	37(25.2%)	0.901	0.637
Age(years)	<50	37(21.8%)	15(13.8%)	10(33.3%)	6.300	0.043
	≥50,<60	23(14.6%)	17(35.4%)	32(29.4%)	13.080	0.001
	≥60	33(17.5%)	10(18.2%)	15(19.0%)	0.090	0.956

Table 6 Logistic regression analysis of related factors for DR (BMI<28kg/m^2, ≥28kg/m^2)

	Factors		B	SE	Wald χ^2	P value	OR	95%CI
BMI<28kg/m^2	Sex		0.168	0.290	0.334	0.563	1.183	0.670-2.089
	Hypertension		0.138	0.213	0.421	0.517	1.148	0.756-1.744
	Drinking history		-0.015	0.279	0.003	0.958	0.986	0.571-1.701
	HbA1c		0.001	0.045	0.000	0.9983	1.001	0.917-1.093
	Family history of DM		-0.406	0.208	3.815	0.051	0.666	0.443-1.001
	Age(years)				1.535	0.464		
		<50	-	-	-	-	1	-
		50~60	0.071	0.279	0.065	0.799	1.074	0.621-1.856
		≥60	-0.221	0.281	0.621	0.431	0.801	0.462-1.390

	Smoking status			1.595	0.450		
	Non smoking	-	-	-	-	1	-
	Light and moderate smoking	0.380	0.328	1.345	0.246	1.463	0.769-2.781
	Heavy smoking	0.342	0.321	1.136	0.286	1.408	0.751-2.640
BMI≥28kg/m^2	Sex	0.040	0.392	0.010	0.919	1.040	0.483-2.242
	Hypertension	0.400	0.318	1.578	0.209	1.492	0.799-2.784
	Drinking history	0.593	0.384	2.378	0.123	1.809	0.852-3.842
	HbA1c	0.000	0.071	0.000	0.995	1.000	0.869-1.150
	Family history of DM	0.398	0.340	1.374	0.241	1.489	0.765-2.898
	Age(years)			7.195	0.027		
	<50	-	-	-	-	1	-
	50~60	-0.149	0.358	0.173	0.678	0.862	0.427-1.739
	≥60	-1.241	0.474	6.838	0.009	0.289	0.114-0.733
	Smoking status			6.920	0.031		
	Non smoking	-	-	-	-	1	-
	Light and moderate smoking	-0.330	0.435	0.573	0.449	0.719	0.306-1.688
	Heavy smoking	0.797	0.404	3.884	0.049	2.219	1.004-4.90

Discussion:

Summary of results

In the study, among the newly diagnosed T2DM patients 20.3% presented with DR. After analyzing the related factors of DR, our study found that obese patients may have a lower incidence of DR (OR=0.587, p=0.004). Many researchers have shown that duration of DM, blood glucose, blood pressure and blood lipids are important risk factors for DR.[16-18] The duration is the most important factor.[19] The subjects of present study were all newly diagnosed type 2 diabetic patients. we found that DR was related to body mass index,smoking status and age of DM diagnosis . To the best of our knowledge, the present study is the first one that is designed to investigate the correlation between smoking level and DR in newly diagnosed patients with T2DM. The results of the study suggested that heavy smoking was associated with DR in obese (BMI $\geqslant$ 28kg/m^2) newly diagnosed T2DM patients(OR=2.219, p=0.049),and there was a negative correlation between DR and the age of diagnosis of diabetes≥60 years(OR=0.289, p=0.009). Those correlations didn't exist in non-obese (BMI<28kg/m^2) patients.

The relationship between BMI and DR

In the study, we found that DR was negatively correlated with BMI. This was not consistent with other research results. Gray et al. followed up 14,657 diabetic patients for an average of 6.68 years and found that increased risk of DR was associated with higher BMI.[20] It had also been reported that BMI was not correlated or even negatively correlated with DR.[21] The subjects in our study had newly diagnosed type 2 diabetes mellitus. The average age of newly T2DM patients with obesity (BMI≥28kg/m^2) was 50.4$\pm$12.4 years, while that of patients without obesity was 55.2$\pm$10.5 years. The former was significantly lower than the latter (t=-6.184, p<0.001). As the onset of T2DM is insidious, patients may have had a long history before diagnosis.[3] Based on the usual clinical experience and their different diagnostic ages, our study suggested that the diagnostic age of T2DM patients with obesity might be closer to the real age of onset. Perhaps because non-obese patients might be confused with more complex diabetic course factor, we did not find any factors in the analysis of the related factors of DR in these patients. In conclusion, we don't think that high BMI is a protective related factor for DR. It is very likely that there are confounding factors, such as an unobserved course of T2DM.

The relationship between smoking and DR

Cigarette smoking is a well-known risk factor for many chronic diseases, including cardiovascular diseases and various malignancies.[6] Many researchers have reported the negative effects of smoking on DM.[22-23] A 14-year prospective cohort study in South Korea showed that the risk of DM among men and women who smoked 20 or more cigarettes a day was 1.55 times higher than that in non-smokers.[7] Cigarette smoking is independently associated with the incidence of T2DM.[24] Although the mechanisms involved are not clear yet. Smoking is associated with insulin resistance, inflammation and dyslipidemia.[25-26] When BMI was ≥28 kg/m^2, we found that the incidence of DR was 12.4% in non-smokers, 12.1% in light and moderate smokers, and 28.2% in heavy smokers (χ^2=11.163,p=0.004).Some studies have reported that no association was found between smoking and DR in patients with T2DM.[27-28]Smoking cessation is one of the important targets for diabetes control and the prevention of chronic diabetes complications.[30]

Studies have shown that cigarette smoking impairs nitric oxide-mediated endothelial function via increased generation of superoxide anions, which may increase the risk of diabetic retinopathy in diabetic patients and could cause eye tissue ischemia, eye tissue hypoxia, retinal arteriosclerosis and decrease of choroidal blood flow, eventually leading to retinal ischemia.[30-31] When BMI was ≥28 kg/m^2, present study shows that the incidence of DR was 28.2% in heavy smokers. Heavy smoking was significantly associated with DR.Smoking may aggravate retinopathy damage by increasing arteriosclerosis.[32] Diabetic retinopathy remains the leading cause of acquired blindness in working-age adults.[4] While the cutting-edge research in the field has identified many molecular, functional, and structural abnormalities, the exact molecular mechanism of this devastating disease remains obscure.[33] A diabetic environment drives the dysfunction of the power generator of the cell and disturbs the homeostasis of the mitochondrial dynamic. Mitochondria appear to have a significant role in the development of diabetic retinopathy, and unraveling the mechanism responsible for their damage as well as the role of epigenetic modifications in mitochondrial homeostasis should identify novel therapeutic targets.[33] Further research is needed to determine whether smoking affects mitochondrial DNA function.

In the present study, DR was found in 20.3% of newly diagnosed type 2 diabetic patients. This was consistent with other findings.[3]The study suggests that smoking may be an important related factor for the occurrence of DR in newly diagnosed diabetic patients. Tyrberg M et al. investigated 794 patients diagnosed with diabetes in 1987-1988 and found that smoking history could increase the risk of diabetic retinopathy during the 9 to 17 years after diagnosis.[34] Because smoking behavior occurs before the diagnosis of T2DM in this study, heavy smoking is very likely to be a risk factor for DR. When BMI was ≥28 kg/m^2, there was a negative correlation between DR and the age of diagnosis of diabetes≥60 years(OR=0.289, p=0.009). Wong et al. investigated more than 1400 patients with type 2 diabetes, and found that the risk of DR in young patients

was significantly higher than that in old patients.[35] After adjusting for factors such as the course of diabetes, the risk of DR was increased twice in those with type 2 diabetes who were less than 45 years old.[35] Delaying the age of diabetes might prevent the occurrence of DR.

Limitations of the study

The study is a single-center cross-sectional study. The results only show the correlation between smoking and diabetic retinopathy. Prospective cohort studies are needed to confirm whether there is a causal relationship between smoking and DR in newly diagnosed T2DM patients. Because of the large number of newly diagnosed type 2 diabetes patients, we have set relatively wide exclusion criteria for the population, which may affect external validity. Although our department is responsible for the first diagnosis and identification of diabetes in Tianjin, it is still unable to achieve a timely diagnosis for all those patients who newly develop diabetes. Because the patients are all from Tianjin, China, the conclusion has got regional limitations.

Conclusion

The study found that heavy smoking and the age of T2DM diagnosis were related factors for the occurrence of DR in newly diagnosed patients when BMI was ≥28 kg/m^2. Although the subjects were with all newly diagnosed patients, it was known that the onset of type 2 diabetes mellitus had been relatively insidious. It is of great importance for people in populations at high-risk of diabetes mellitus to quit smoking. Delaying the age of diabetes may prevent the occurrence of DR. Therefore, long-term cohort studys were needed to elucidate the correlation.

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Contributors: Zhaohu Hao wrote the manuscript. Xiao Huang was in charge of fundus

examination. Yongzhang Qin was responsible for basic information collection. Hailin Shao was responsible for the statistical analysis of the data. Huanming Li was responsible for auditing the fundus images and reports. Rong Xu was responsible for keeping patients in order. Baocheng Chang proposed the necessity and design of the study. Fengshi Tian was the director of our department and also provided project support for the research.

Data sharing statement: Data is available upon reasonable request.

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Analysis of factors related to diabetic retinopathy in newly diagnosed type 2 diabetic patients: a cross-sectional study

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Abstract

Aim: To investigate the related factors of diabetic retinopathy (DR) and explore the correlation between smoking and DR in patients with newly diagnosed type 2 diabetes.

Design: A single-center cross-sectional study

Setting: Tianjin 4th Central Hospital

Participants: Patients with newly diagnosed type 2 diabetes who visited the outpatient department of the hospital from December 2018 to April 2019

Methods: A total of 947 patients were enrolled in the study. They were divided into two groups according to whether they were diagnosed with DR (diabetic retinopathy group, DR group; non-diabetic retinopathy group, NDR group). The smoking index (SI) was calculated to assess smoking status. Factors such as sex, age, hypertension, T2DM diagnosed age, family history of diabetes, drinking history, HbA1c, body mass index (BMI) and smoking status were compared between the two groups. Logistic regression was used to analyze the relationship between DR and the above factors.

Results There was no statistically significant difference between the two groups in sex, age, hypertension, DM diagnosed age, family history of diabetes, drinking history and HbA1c. BMI was significantly higher in DR patients (27.7 ± 4.2vs26.7 ± 4.4, p=0.004). Smoking status was also different between the two groups (χ^2=6.350, p=0.042). BMI was shown to be a related factor for DR in newly diagnosed diabetic patients (OR=0.592, p=0.004). When BMI was ≥28 kg/m^2, heavy smoking was significantly associated with DR (OR=2.219, p=0.049), and there was a negative correlation between DR and the age of diagnosis of diabetes ≥60 years (OR=0.289, p=0.009).

Conclusions Heavy smoking was an important related factor for DR in newly diagnosed diabetes mellitus patients when BMI was ≥28 kg/m^2. Delaying the age of diabetes might prevent the occurrence of DR. To elucidate the correlation, long-term cohort studies with large samples are needed.

Keywords: Smoking index, Type 2 diabetes mellitus, Diabetic retinopathy, Newly diagnosed

diabetes mellitus.

Strengths and limitations of this study

This study is a representative population-based study.

There are few studies on the factors related to newly diagnosed diabetic retinopathy in type 2 diabetes mellitus patients in China.

This study was the first to use the smoking index to assess smoking severity to study diabetic retinopathy.

The study is a single-center cross-sectional study.

Introduction

Diabetes mellitus (DM) is a chronic metabolic disease characterized by hyperglycemia. It has various etiologies, and the incidence is increasing yearly worldwide. According to the report of the International Diabetes Federation (IDF) in 2017, about 425 million people were affected by DM, and that number is expected to reach 629 million by 2045.[1] China has the highest number of DM patients, and the prevalence has increased from 0.67% in 1980 to 10.4% in 2013.[2] A epidemiological-based meta-analysis conducted in 35 research centers around the world showed that the prevalence of DR in the total diabetic population was 35.4%.[3] DR is a highly specific microvascular complication caused by diabetes, and its prevalence is significantly correlated with the course of DM and blood glucose levels.[4] DR is the most important cause of irreversible

blindness in working-age adults.[4] Chronic hyperglycemia causes not only retinal vascular diseases but also damage to retinal neurons, both of which are factors that lead to vision loss.[5] Twenty percent of newly diagnosed type 2 diabetic (T2DM) patients are diagnosed with DR.[3] The explanation for this phenomenon is that the onset of T2DM is often insidious, and patients may have a long history of untreated hyperglycemia before diagnosis.[3] Are there any other risk factors for DR in these newly diagnosed patients? There is no relevant research report at present.

Smoking is harmful to health, and it is an important risk factor for cardiovascular, cerebrovascular and respiratory diseases and also for malignant tumors.[6] Smoking is one of the most serious public health issues throughout the world, and it is responsible for about 7 million deaths per year worldwide. [6] In a 14-year prospective cohort study, Jee SH found that people smoking more than 20 cigarettes a day had a 1.55-fold higher incidence of diabetes than non-smokers.[7] It is well-known that smoking may cause an increase in proteinuria.[8] However, many studies have shown that smoking has no significant correlation with DR.[9] Even some studies in China have shown that smoking is a protective factor for DR.[10] Many diabetic patients had started smoking before they were diagnosed with diabetes. Does smoking contribute to the complications of DR in newly diagnosed patients? Little research has been done on the relationship between smoking and DR in newly diagnosed diabetic patients. The present study investigated the factors related to DR and explored the correlation between smoking and DR in patients with newly diagnosed T2DM who visited the outpatient department of the hospital from December 2018 to April 2019.

Research design and methods

The present study was a single-center cross-sectional study of factors associated with DR in newly diagnosed T2DM patients in Tianjin 4th Central Hospital. The hospital is a regional medical center in Tianjin. The hospital had a diabetes identification center. The patients in the region are identified by the department before they can enjoy more preferential health insurance policies in the clinic. The Metabolic Disease Management Center (MMC) was responsible for the screening for complications in newly diagnosed diabetes patients, such as urinary protein, fundus examination, peripheral vascular and neuropathy. The diabetes nursing team undertook the measurement of height, weight and blood pressure, and gathered information about smoking, drinking and past disease history. Electronic medical records were generated at the same time.

Participants: Study participants were newly diagnosed type 2 diabetic patients who visited MMC from December 2018 to April 2019. The patient information came from an electronic database of the hospital's diabetes identification center. All the patients who underwent fundus examination were enrolled in this study. All patient identifiers were removed prior to analysis. This clinical study protocol was approved by the institutional review board of Tianjin 4th Central Hospital, and all steps were conducted in accordance with the principles of the World Medical Association Declaration of Helsinki[11] (Trial registration code: ChiCTR1900020728). Written informed consent was obtained from each patient.

Inclusion criteria

The study enrolled participants with a baseline of being newly diagnosed with T2DM within the past six months; patients who could communicate independently and describe their smoking status; none of the smokers had given up smoking before the study; and all patients underwent DR screening in the study.

According to the Chinese guidelines for the prevention and treatment of diabetes, all patients with diabetes in our hospital are required to be diagnosed with an oral glucose tolerance test. The diagnostic criteria during execution were as follows: (1) Fasting plasma glucose (FPG) ≥7.0 mmol/l. Fasting was defined as no caloric intake for at least 8 h; or (2) 2-h plasma glucose ≥11.1 mmol/l during an oral glucose tolerance test (OGTT). The test was performed as described by the World Health Organization (WHO), using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water. A HbA1c test was not standardized in China, so it could not be used as a diagnostic standard.[12]

Exclusion criteria

Type 1 diabetes and other specific types of diabetes; acute complications of diabetes, such as diabetic ketoacidosis or lactic acidosis; hypovolemia; subjects with diabetic nephropathy complications, rheumatic diseases, autoimmune diseases, pregnancy, malignant tumors, infection, foot ulcer, severe mental illness, thyroid dysfunction, severe hepatic insufficiency or renal insufficiency, severe anemia, heart failure or respiratory insufficiency; cognitive impairment and communication disorders.

Evaluation of clinical variables

Information about the patients' names, sex, phone numbers, BMI, age, smoking history, drinking history, family history of DM, hypertension and HbA1c was collected using a uniform information table. The formula for BMI was weight in kilograms divided by the square of height in meters. BMI (≥28 kg/m^2) met obesity criteria according to Chinese standards.[13] Venous blood samples were collected in EDTA tubes from fasting patients in the morning. The level of HbA1c was determined by affinity chromatography in the hospital standard laboratory (**Tosoh Corporation, Japan**). Smoking status assessment: According to the WHO (1997) smoking survey method, smoking at least one cigarette per day for more than six months was considered as a smoking history. [14] The smoking index (SI) was calculated by multiplying the number of cigarettes smoked per day by the number of years the person had smoked. In our study, according to the SI, 0 meant non-smoking, ≤500 meant light and moderate smoking, and > 500 meant heavy smoking.

Screening for DR was carried out according to EURODIAB guidelines using one 45° field retinograph centered on the fovea. If DR was suspected, other two 45°retinographs were obtained.[15] Ophthalmologists judged whether the patient had DR according to the results of the funduscopic examination and guidelines. In the study, the results were reported as either positive or negative (DR or NDR), and the patients were divided into two groups (DR group and NDR group). Non-diabetic retinopathy here included a normal fundus and other retinopathy that may be caused by other causes (non-diabetic) such as fundus arteriosclerosis.

Statistical analysis

Descriptive analysis was used to illustrate the basic demographic characteristics. For continuous variables, a one-sample Kolmogorov-Smirnov normality test was used to check the normality of the distribution of such variables. Once normal distribution and homogeneity of variance were satisfied, values were represented by the $\bar{x} \pm SD$ and Student's t-test was used. For categorical variables, a χ^2 test was performed. The occurrence of DR was identified as the dependent variable, and the related factors (sex, hypertension, age at DM diagnosis, family history of diabetes, drinking history, HbA1c, BMI and smoking status) were listed as independent variables. To analyze the factors associated with DR and adjust for potential confounding effects, we examined the factors associated with DR using multivariable logistic regression analysis. All the analyses conducted using SPSS 16.0 software with $p < 0.05$ (two-sided) indicated statistical significance.

Patient and Public Involvement

Patients were not involved in setting the research questions or planning the study. Investigators did not know the identities of the study participants. In this study, the electronic data were obtained from the health records at the institution. All patient identifiers were removed before the analysis was conducted. There was no direct patient or public involvement.

Results

Characteristics of the study population

In this survey, 952 patients entered the study initially and 5 were excluded because their fundus could not be seen clearly. Finally, a total of 947 patients were enrolled in the study. Their average age was 53.2±11.6 years. The average HbA1c level was 9.53±2.13%. The average BMI of the patients was 27.5±4.3 kg/m^2. A total of 430 patients had a history of smoking. The average smoking age was 27.8±12.2 years (2–60 years). They smoked 21.8±12.3 cigarettes per day. Baseline characteristics are shown in Table 1.

Comparison of clinical data between the DR and NDR groups

A total of 755 patients (79.7%) were in the NDR group, and 192 patients were in the DR group. Among the patients in the DR group, there were 12 patients with PDR. The comparison of clinical data between the DR and NDR groups is shown in Table 2. There were no statistically significant differences between the two groups in sex, age, hypertension, family history of diabetes, drinking history and HbA1c. The BMI was significantly higher in NDR patients (27.7±4.2 vs 26.7 ±4.4, p=0.004). Smoking status was different between the two groups (χ^2=6.350, p=0.042).

The occurrence of DR was identified as the dependent variable, and the related risk factors were listed as independent variables. Both of the variables were progressed through multivariate logistic regression analysis; age, BMI and smoking status were adjusted, as shown in Table 3. Patients with BMI ≥28 kg/m^2 were less likely to have DR in newly diagnosed diabetic patients

(OR=0.592, p=0.004). The age of DM diagnosis was also statistically significant in the regression analysis (p=0.047)**.** The incidence of DR in patients over 60 years who were diagnosed with diabetes was significantly lower than that in patients under 50 years (OR=0.596, p=0.024). The incidence of DR in severe smokers was significantly higher than that in nonsmokers (OR=1.664, p=0.043).

Did obesity have a protective effect on DR?

Our study compared the age, HbA1c, smoking and drinking history, family history of DM and complications between the obese and non-obese patients, as shown in Table 4 (BMI ≥28 kg/m^2 vs BMI <28 kg/m^2). There was no statistically significant difference between the two groups in sex, family history of DM, drinking history, smoking history and HbA1c. The age of obese diabetic patients was significantly lower than that of non-obese patients (50.4±12.4 vs 55.2±10.5, t=-6.184, p<0.001). Because the onset of T2DM was insidious, patients might have a long history before diagnosis.[3] BMI could be a possible confounding variable.

After adjustment for BMI and age, DR-related factors were further analyzed. First, the participants were divided into two groups according to whether the BMI was ≥28 kg/m^2. The incidence of DR in patients with different smoking statuses was compared (Table 5). The incidence of DR in heavy smoking patients was significantly higher in obese patients. There was no significant difference in the incidence of DR with different smoking statuses among patients over 60 years (Table 5). Then after adjustment for age, multiple logistic regression analysis was used to analyze the related factors of DR (Table 6). When BMI was ≥28 kg/m^2, heavy smoking was significantly associated with DR (OR=2.219, p=0.049), and there was a negative correlation

between DR and the age of diagnosis of diabetes at ≥60 years (OR=0.289, p=0.009).

Table 1 Baseline characteristics of 947 participants with newly diagnosed T2DM.

Items	n	%
Sex		
male	566	59.8
female	381	40.2
With family history of DM	580	61.2
Age		
< 50 years old	309	32.5
≥50, < 60 years old	318	33.4
≥60 years old	325	34.1
With hypertension	498	52.6
With drinking history	195	20.6
Smoking status		
Non-smoking	517	54.6
Light and moderate smoking	212	22.4
Heavy smoking	218	23.0

Abbreviations: DM, Diabetes mellitus.

Table 2 Comparison of clinical data between DR and NDR groups

Items		NDR (n=755)	DR (n=192)	t/χ^2	P value
Sex	Male	447(79.0%)	119(21.0%)	0.490[a]	0.510
	Female	308(80.8%)	73(19.2%)		
Age	years	53.3±11.7	52.9±11.1	0.350[b]	0.726
Family history of DM	no	288(78.5%)	79(21.5%)	0.580[a]	0.456
	yes	467(80.5%)	113(19.5%)		
Drinking history	no	608(80.9%)	144(19.1%)	2.863[a]	0.109
	yes	147(75.4%)	48(24.6%)		
Hypertension[c]	no	349(81.2%)	81(18.8%)	1.457[a]	0.265
	yes	392(78.7%)	106(21.3%)		
Age of DM diagnosis	< 50 years	247(79.9%)	62 (20.1%)	2.382[a]	0.304

	≥50, < 60 years	243(77.1%)	72(22.9%)		
	≥60 years	265(82.0%)	58(18.0%)		
HbA1c	%	9.51±2.13	9.60±2.12	-0.546[b]	0.585
BMI	kg/m^2	27.7±4.2	26.7±4.4	2.921[b]	0.004
Smoking history	no	424(82.0%)	93(18.0%)	3.682[b]	0.062
	yes	331(77.0%)	99(23.0%)		
Smoking status	Non-smoking	424(82.0%)	93(18.0%)	6.350[a]	0.042
	Light and moderate smoking	170(80.2%)	42(19.8%)		
	Heavy smoking	161(73.9%)	57(26.1%)		

Note: [a] Chi-squared test, [b] Student's t test and [c]19 cases of patients with missing data. DR, diabetic retinopathy; NDR, non-diabetic retinopathy; HbA1c, hemoglobin A1c; BMI, body mass index; DM, diabetes mellitus. There was no statistically significant difference if p >0.05.

Table 3 Analysis of related factors with DR, adjusted for age, BMI and smoking status.

Factors	B	SE	Wald χ^2	P value	OR	95%CI
Sex	0.099	0.228	0.190	0.663	1.104	0.707–1.726

Hypertension	0.252	0.175	2.080	0.149	1.287	0.914–1.812
Drinking history	0.131	0.222	0.351	0.554	1.140	0.739–1.761
HbA1c	0.006	0.038	0.022	0.882	1.006	0.934–1.083
BMI (≥28 kg/m^2 vs <28 kg/m^2)	-0.524	0.183	8.230	0.004	0.592	0.414–0.847
Family history of DM	-0.162	0.173	0.877	0.349	0.850	0.606–1.194
Age of DM diagnosis (years)			6.126	0.047		
<50	-	-	-	-	1	-
50~60	-0.092	0.216	0.183	0.669	0.912	0.597–1.393
≥60	-0.518	0.229	5.124	0.024	0.596	0.381–0.933
Smoking status			4.658	0.097		
Non smoking	-	-	-	-	1	-
Light and moderate smoking	0.097	0.252	-0.149	0.700	1.102	0.673–1.806
Heavy smoking	0.509	0.251	4.104	0.043	1.664	1.017–2.724
Constant	-1.407	0.574	6.016	0.014	0.245	

Abbreviations: DR, diabetic retinopathy; HbA1c, hemoglobin A1c; BMI, Body mass index; DM, diabetes mellitus.

Table 4 Comparison of clinical data between patients (BMI ≥28 kg/m^2 vs BMI <28k g/m^2)

Items		BMI≥28 kg/m^2 (n=379)	BMI<28 kg/m^2 (n=573)	t/χ^2	P value
Sex (n, %)	Male	230(60.7%)	338(59.0%)	0.273[a]	0.637
	Female	149(39.3.8%)	235(41.0%)		
Age	years	50.4±12.4	55.2±10.5	-6.184[b]	<0.001
Family history of DM	no	141(37.2%)	228(39.8%)	0.643[a]	0.455
(n, %)	yes	238(62.8%)	345(60.2%)		
Drinking history (n, %)	no	305(80.5%)	452(78.9%)	0.567[a]	0.305
	yes	74(19.5%)	121(21.1%)		
Hypertension[c] (n, %)	no	148(40.1%)	284(50.4%)	9.419[a]	0.002
	yes	221(59.9%)	280(49.6%)		
HbA1c	%	9.38±2.10	9.63±2.30	-1.744[b]	0.082
Smoking history (n, %)	no	217(57.3%)	303(52.9%)	1.763[b]	0.206
	yes	162(42.7%)	270(47.1%)		

Note: [a] Chi-squared test, [b] Student's t test and [c]19 cases of patients with missing data. There was no statistically significant difference if p >0.05. HbA1c, hemoglobin A1c; BMI, Body mass index; DM, diabetes mellitus.

Table 5 The incidence of DR in patients with different smoking statuses

Items		Non-smoking (n, %)	Light and moderate smoking (n, %)	Heavy smoking (n, %)	χ^2	P value
BM I(kg/m^2)	≥28	27(12.4%)	11(12.1%)	20(28.2%)	11.163	0.004
	<28	66(22.0%)	31(25.6%)	37(25.2%)	0.901	0.637
Age(years)	<50	37(21.8%)	15(13.8%)	10(33.3%)	6.300	0.043
	≥50, <60	23(14.6%)	17(35.4%)	32(29.4%)	13.080	0.001
	≥60	33(17.5%)	10(18.2%)	15(19.0%)	0.090	0.956

Abbreviations: DR, diabetic retinopathy; BMI, body mass index.

Table 6 Logistic regression analysis of related factors for DR (BMI <28 kg/m^2, ≥28 kg/m^2)

	Factors	B	SE	Wald χ^2	P value	OR	95%CI
BMI <28 kg/m^2	Sex	0.168	0.290	0.334	0.563	1.183	0.670–2.089

	Hypertension	0.138	0.213	0.421	0.517	1.148	0.756–1.744
	Drinking history	-0.015	0.279	0.003	0.958	0.986	0.571–1.701
	HbA1c	0.001	0.045	0.000	0.9983	1.001	0.917–1.093
	Family history of DM	-0.406	0.208	3.815	0.051	0.666	0.443–1.001
	Age (years)			1.535	0.464		
	<50	-	-	-	-	1	-
	50~60	0.071	0.279	0.065	0.799	1.074	0.621–1.856
	≥60	-0.221	0.281	0.621	0.431	0.801	0.462–1.390
	Smoking status			1.595	0.450		
	Non smoking	-	-	-	-	1	-
	Light and moderate smoking	0.380	0.328	1.345	0.246	1.463	0.769–2.781

	Heavy smoking	0.342	0.321	1.136	0.286	1.408	0.751–2.640
BMI ≥28 kg/m^2	Sex	0.040	0.392	0.010	0.919	1.040	0.483–2.242
	Hypertension	0.400	0.318	1.578	0.209	1.492	0.799–2.784
	Drinking history	0.593	0.384	2.378	0.123	1.809	0.852–3.842
	HbA1c	0.000	0.071	0.000	0.995	1.000	0.869–1.150
	Family history of DM	0.398	0.340	1.374	0.241	1.489	0.765–2.898
	Age (years)			7.195	0.027		
	<50	-	-	-	-	1	-
	50~60	-0.149	0.358	0.173	0.678	0.862	0.427–1.739
	≥60	-1.241	0.474	6.838	0.009	0.289	0.114–0.733

Smoking status			6.920	0.031		
Non smoking	-	-	-	-	1	-
Light and moderate smoking	-0.330	0.435	0.573	0.449	0.719	0.306–1.688
Heavy smoking	0.797	0.404	3.884	0.049	2.219	1.004–4.903

Abbreviations: DR, diabetic retinopathy; HbA1c, hemoglobin A1c; BMI, body mass index; DM, diabetes mellitus.

Discussion

Summary of results

In our study, among the newly diagnosed T2DM patients, 20.3% presented with DR. After analyzing the related factors of DR, the present study found that obese patients may have a lower incidence of DR (OR=0.592, p=0.004). Many researchers have shown that duration of DM, blood glucose, blood pressure and blood lipids are important risk factors for DR.[16-18] The duration is the most important factor.[19] The subjects of the present study were all newly diagnosed T2DM patients. Our study showed that DR was related to BMI, smoking status and age of DM diagnosis. To the best of our knowledge, the present study is the first that was designed to investigate the correlation

between smoking level and DR in newly diagnosed patients with T2DM. The results of the study suggested that heavy smoking was associated with DR in obese (BMI ⩾28 kg/m^2) newly diagnosed T2DM patients (OR=2.219, p=0.049), and there was a negative correlation between DR and the age of diagnosis of diabetes ≥60 years (OR=0.289, p=0.009). Those correlations did not exist in non-obese (BMI <28 kg/m^2) patients.

The relationship between BMI and DR

In our study, DR was negatively correlated with BMI. This was not consistent with other research results. Gray et al. followed up with 14, 657 diabetic patients for an average of 6.68 years and found that increased risk of DR was associated with higher BMI.[20] It has also been reported that BMI was not correlated or even negatively correlated with DR.[21] The subjects in our study had newly diagnosed T2DM. The average age of newly T2DM patients with obesity (BMI ≥28 kg/m^2) was 50.4±12.4 years, while that of patients without obesity was 55.2±10.5 years. The former was significantly lower than the latter (t=-6.184, p<0.001). As the onset of T2DM is insidious, patients may have had a long history before diagnosis.[3] Based on the usual clinical experience and their different diagnostic ages, our study suggested that the diagnostic age of T2DM patients with obesity might be closer to the real age of onset. Perhaps because non-obese patients might be confused with more complex diabetic course factors, we did not find any factors in the analysis of the related factors of DR in these patients. In conclusion, we do not think that a high BMI is a protective related factor for DR. It is very likely that there are confounding factors, such as an unobserved course of T2DM.

The relationship between smoking and DR

Cigarette smoking is a well-known risk factor for many chronic diseases, including cardiovascular diseases and various malignancies.[6] Many researchers have reported the negative effects of smoking on DM.[22-23] A 14-year prospective cohort study in South Korea showed that the risk of DM among men and women who smoked 20 or more cigarettes a day was 1.55 times higher than that in non-smokers.[7] Cigarette smoking is independently associated with the incidence of T2DM.[24] Although the mechanisms involved are not clear yet. Smoking is associated with insulin resistance, inflammation and dyslipidemia.[25-26] When BMI was ≥28 kg/m^2, we found that the incidence of DR was 12.4% in non-smokers, 12.1% in light and moderate smokers and 28.2% in heavy smokers (χ^2=11.163, p=0.004). Some studies have reported that no association was found between smoking and DR in patients with T2DM.[27-28]Smoking cessation is one of the important targets for diabetes control and the prevention of chronic diabetes complications.[29-30]

Studies have shown that cigarette smoking impairs nitric oxide-mediated endothelial function via increased generation of superoxide anions, which may increase the risk of DR in diabetic patients and could cause eye tissue ischemia, eye tissue hypoxia, retinal arteriosclerosis and a decrease in choroidal blood flow, eventually leading to retinal ischemia.[30-31] When BMI was ≥28 kg/m^2, the present study showed that the incidence of DR was 28.2% in heavy smokers. Heavy smoking was significantly associated with DR. Smoking may aggravate retinopathy damage by increasing arteriosclerosis.[32] DR remains the leading cause of acquired blindness in working-age adults.[4] While cutting-edge research in the field has identified many molecular, functional and structural abnormalities, the exact molecular mechanism of this devastating disease remains obscure.[33] A diabetic environment drives the dysfunction of the power generator of the cell and

disturbs homeostasis of the mitochondrial dynamic. Mitochondria appear to have a significant role in the development of DR, and unraveling the mechanism responsible for their damage as well as the role of epigenetic modifications in mitochondrial homeostasis should identify novel therapeutic targets.[33] Further research is needed to determine whether smoking affects mitochondrial DNA function.

In the present study, DR was found in 20.3% of newly diagnosed T2DM patients. This was consistent with other findings.[3] The study suggested that smoking may be an important related factor for the occurrence of DR in newly diagnosed diabetic patients. Tyrberg M et al. investigated 794 patients diagnosed with diabetes in 1987–1988 and found that smoking history could increase the risk of DR during 9–17 years after diagnosis.[34] Because smoking behavior occurred before the diagnosis of T2DM in this study, heavy smoking is very likely to be a risk factor for DR.

When BMI was ≥28 kg/m^2, there was a negative correlation between DR and the age of diagnosis of diabetes ≥60 years (OR=0.289, p=0.009). Wong et al. investigated more than 1400 patients with T2DM and found that the risk of DR in young patients was significantly higher than that in old patients.[35] After adjusting for factors such as the course of diabetes, the risk of DR was increased twice in those with T2DM who were less than 45 years.[35] Our report agrees with other studies. Age at onset of diabetes was strongly associated with an increasing number of complications.[36] In other regions, studies that have looked at age of onset of diabetes have found that early onset diabetes increases disease severity. Genetic determinants of diabetes are likely to be more common in patients with early onset of diabetes.[37]This observation suggests that

patients with early onset of diabetes have a more aggressive disease and are prone to develop more complications at an earlier age.[38]Delaying the age of diabetes might prevent the occurrence of DR.

Limitations of the study

The study was a single-center cross-sectional study. The results only showed a correlation between smoking and DR. Prospective cohort studies are needed to confirm whether there is a causal relationship between smoking and DR in newly diagnosed T2DM patients. Because of the large number of newly diagnosed T2DM patients, we have set relatively wide exclusion criteria for the population, which may affect external validity. Although our department is responsible for the first diagnosis and identification of diabetes in Tianjin, it is still unable to achieve a timely diagnosis for all those patients who newly develop diabetes. Because the patients are all from Tianjin, China, the conclusion has regional limitations.

Conclusion

The study found that heavy smoking and the age of T2DM diagnosis were related factors for the occurrence of DR in newly diagnosed patients when BMI was ⩾28 kg/m². Although the subjects were all newly diagnosed patients, it is known that the onset of T2DM is relatively insidious. It is of great importance for people in populations at high-risk of diabetes mellitus to quit smoking. Delaying the age of diabetes may prevent the occurrence of DR. Therefore, long-term cohort studies are needed to elucidate the correlation.

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