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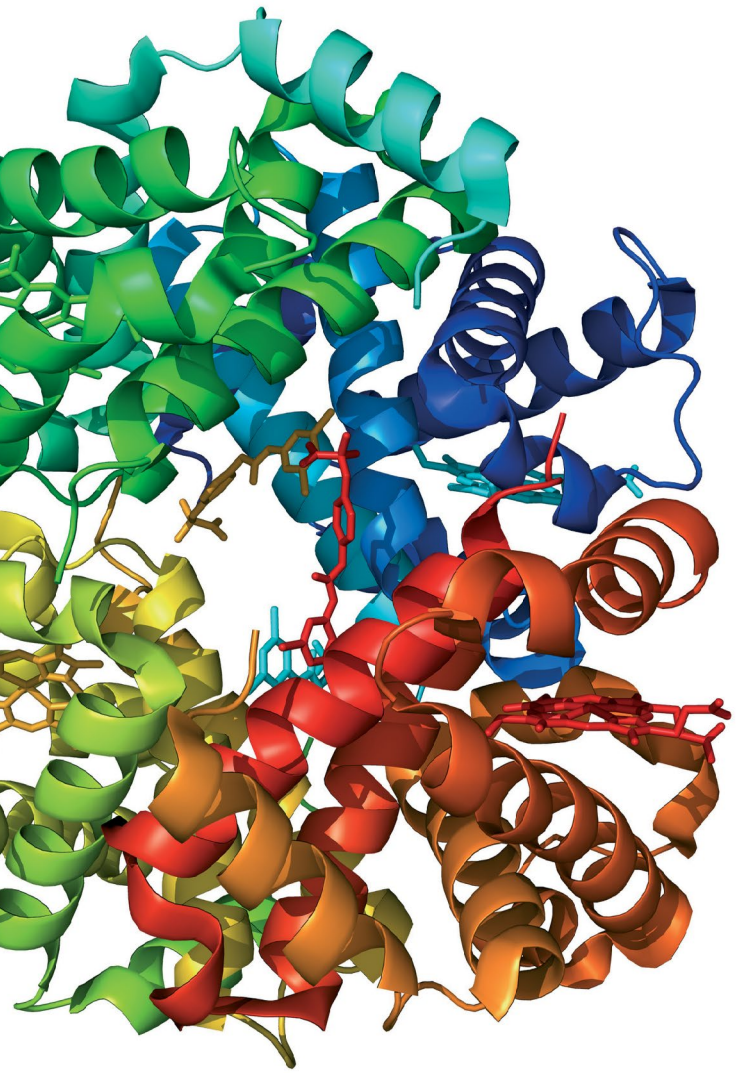

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Platelet volume indices: markers of carotid atherosclerosis in type 2 diabetes mellitus?

ABSTRACT

Background. Platelet volume indices (PVI) such as mean platelet volume (MPV), platelet distribution width (PDW), and platelet large cell ratio (P-LCR) are the indicators of platelet activity and may have a role in subclinical atherosclerosis and microvascular complications in type 2 diabetes mellitus (T2DM). We evaluated PVI in diabetics for their association with carotid intima media thickness (CIMT) and microvascular complications.

Methods. Participants — 105 T2DM patients and age, gender matched 105 controls were evaluated by history and complete blood counts (CBC) including PVI, blood sugars, HbA_{1c}, lipid profile and microvascular complications. PVI were compared between cases and controls. Carotid Doppler was done and CIMT was correlated with PVI.

Results. PVI were found significantly higher in diabetic patients compared to controls. Mean MPV in cases *vs.* controls was (11.09 ± 1.02 fL *vs.* 10.28 ± 0.96 fL, $p \leq 0.001$), mean PDW (13.46 ± 1.96 fL *vs.* 12.85 ± 3.54 fL, $p = 0.12$), mean P-LCR (31.92 ± 6.23% *vs.* 27.94 ± 5.94%, $p \leq 0.001$). CIMT showed a positive significant association with MPV, PDW and PLCR, dyslipidemia and negative with glycemic control. PVI, especially MPV was significantly elevated in those with neuropathy, nephropathy and retinopathy.

Conclusion. PVI i.e. MPV, PDW, P-LCR are increased in diabetic patients. They correlate positively with CIMT, implying cardiovascular risk. PVI have a positive association with microvascular complications also. PVI as determined by simple automated CBC can be used as markers of subclinical atherosclerosis and predictor of future cardiovascular events in T2DM. (Clin Diabetol 2020; 9; 2: 103–111)

Key words: T2DM, PVI, increased CIMT, microvascular complications

Introduction

In T2DM, platelets and their interaction with the vessel wall play a role in atherogenesis and in the formation of the coronary thrombus. Several haemorrheological alterations take place in both T1DM and T2DM. The erythrocytes are altered and become less deformable, contributing to increased whole blood viscosity. The platelet function is also altered in diabetes. Diabetic thrombocytopathy refers to differences in platelet function between diabetic and nondiabetic individuals. Among diabetic individuals, differences are due to the following: reduced membrane hydration, altered Ca^{2+} and Mg^{2+} homeostasis (increased intracellular Ca^{2+} mobilization and decreased intracellular Mg^{2+}), increased arachidonic acid metabolism, increased TXA_2 synthesis, decreased prostacyclin production, decreased NO production, decreased antioxidant levels, increased expression of activation-dependent adhesion molecules (e.g., GpIIb–IIIa, P-selectin) [1].

Platelets from patients with type 1 and type 2 diabetes exhibit enhanced platelet aggregation activity early in the disease course that may precede the development of cardiovascular disease (CVD). Numerous biochemical abnormalities have been found that correlate with platelet hyperreactivity. Platelets from diabetic patients exhibit reduced membrane hydration as described above, which may reflect changes in the

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lipid composition of the membrane or glycation of membrane proteins. Arachidonic acid metabolism is increased in platelets from diabetic patients; this leads to enhanced TXA_2 production and may contribute to increased platelet sensitivity. Platelets in type 2 diabetic individuals adhere to vascular endothelium and aggregate more readily than those in healthy people. Loss of sensitivity to the normal restraints exercised by prostacyclin [PGI (2)] and nitric oxide (NO) generated by the vascular endothelium presents as the major defect in platelet function. Insulin is a natural antagonist of platelet hyperactivity. It sensitizes the platelet to PGI (2) and enhances endothelial generation of PGI (2) and NO. Thus, the defects in insulin action in diabetes create a milieu of disordered platelet activity conducive to macrovascular and microvascular events [2]. Platelet size is also increased in diabetes. The large platelets having dense granules are more active biochemically, functionally, and metabolically, have higher thromboxane A2 levels, express more glycoprotein Ib and IIb/IIIa receptors, and could be a risk factor for developing coronary thrombosis [3]. Patients with type 2 diabetes mellitus, without proven vascular disease, exhibit platelet dysfunction and have increased platelet aggregation and aspirin insensitivity compared to non-diabetic patients with previous MI [4].

Many researchers have found an association between mean platelet volume, cardiovascular events like acute MI and glycemic status. There are reports of negative association also. Hence, we wanted to explore the relation between T2DM and all platelet volume indices and whether these indices which are available as a part of complete blood counts as determined by automated counters, can be used as markers of future cardiovascular events.

The first structural change that can be detected in atherosclerosis is an increase in intima-media thickness (IMT). The value of CIMT as a surrogate endpoint in interventional or observational studies as an alternative to cardiovascular disease (CVD) is strengthened by its associations with risk factors and coronary or cerebrovascular events.

In this study, all components of platelet volume indices were compared with controls to see if they are altered in T2DM and if they are associated with increased CIMT and can be used as marker of subclinical atherosclerosis.

Methods

This hospital-based case control study was carried out in our tertiary care Centre from Januar 2016 to August 2017 after approval from institutional ethical committee.

Study population

105 consecutive patients of T2DM were selected from Diabetes Clinic and Medicine outpatient department. 105 healthy age and gender matched non-diabetic visitors or relatives, or unrelated attendants of hospitalized patients were selected as controls after written informed consent.

Eligibility criteria

Cases: T2DM in age ≥ 30 years diagnosed by ADA criteria.

Patients were excluded if they had diabetes other than T2DM, any acute complications of diabetes, acute infections, critical illness, myeloproliferative disorders, thrombocytopenia due to any other cause e.g. immune thrombocytopenia, hemoglobin < 10 g/dL in females and < 11 g/dL in males, pre-existing peripheral vascular disease, stroke and/or angiographically proven coronary artery disease (CAD) with or without revascularization, or on antiplatelet drugs the controls were age and sex matched healthy subjects. Exclusion criteria for controls were same as those for cases.

Detailed diabetic history with respect to onset, duration, treatment and hypoglycaemic episodes was obtained. Relevant history, personal history, any addictions or drug abuse and socioeconomic data was recorded. Cardiovascular risk factors were evaluated in all patients according to standard definitions. *Smokers included current smokers and ex-smokers of < 12 months, whereas non-smokers included ex-smokers of more than 12 months and never smokers. **Alcoholism was defined as more than 14 drinks per week in men and more than 7 drinks in women. ***Hypertensives included patients on antihypertensive medications or newly detected hypertension as per JNC VIII criteria. #Positive family history was defined as occurrence of CAD or sudden cardiac death in first degree relatives before 40 years of age. ##Dyslipidemia was defined as serum (Sr) cholesterol > 200 mg/dL and/or serum triglycerides > 150 mg/dL and/or serum HDL < 40 mg/dL in males and < 50 mg/dL in females (Table 1).

Physical measurements specifically height, weight, BMI, were recorded and blood pressure measurements, electrocardiogram were done in each patient.

All patients were evaluated for macro and microvascular complications of diabetes. Those with macrovascular complications were excluded. Evaluation for microvascular complications was done as follows — peripheral neuropathy: assessed by Toronto Clinical neuropathy score and SW monofilament test. Diabetic retinopathy was assessed by fundus examination through dilated pupil by ophthalmologist and diabetic nephropathy was assessed by microalbuminuria and eGFR.

Table 1. Baseline characteristics, cardiovascular risk factors and PVI in cases and controls

SN	Parameter	Cases N = 105	Controls N = 105	p-value
1.	Age in years			
	Mean ± SD (range)	55.5 ± 10.3 (31–80)	55.7 ± 10.3 (31–77)	0.927
2.	Gender			
	Males	48 (46%)	48 (46%)	1.00
	Females	57 (54%)	57 (54%)	
3.	Hemoglobin [g/dL] Mean ± SD	12.7 ± 1.34	12.31 ± 0.23	0.73
4.	Total leucocyte Count [10^3/cu mm] Mean ± SD	8.32 ± 2.66	7.8 ± 3.49	0.815

		MPV (fL)	p-value	PDW (fL)	p-value	P-LCR (%)	p-value
5.	Duration of DM						
	< 5 yrs (n = 66)	10.73 ± 0.81	**< 0.05**	12.82 ± 1.41	**< 0.05**	29.51 ± 4.83	**< 0.05**
	≥ 5 yrs (n = 39)	11.73 ± 0.73		14.54 ± 1.41		36.72 ± 3.74	
6.	Glycemic status						
	HbA_{1c} < 7% (n = 22)	10.95 ± 0.69	0.48	12.98 ± 1.37	0.19	30.81 ± 4.54	0.35
	HbA_{1c} ≥ 7% (n = 83)	11.12 ± 1.1		13.59 ± 2.08		32.22 ± 6.64	
7.	Cardiovascular risk factors						
a	Smokers* (n = 24)	11.23 ± 0.99 (9.1–13.6)	0.45	13.54 ± 1.75 (9.6–17.1)	0.82	32.74 ± 6.19 (17.6–43.5)	0.47
	Non smokers (n = 81)	11.05 ± 1.04		13.44 ± 2.03		31.68 ± 6.31	
b	Alcoholics** (n = 18)	11.16 ± 1.2 (9.1–13.6)	0.75	13.26 ± 1.95 (9.6–17.1)	0.63	31.69 ± 6.97 (17.6–43)	0.86
	Non alcoholics (n = 87)	11.07 ± 0.99		13.51 ± 1.97		31.97 ± 6.16	
c	Hypertensives*** (n = 41)	11.31 ± 1.11 (9.1–14)	0.06	13.85 ± 2.23 (9.6–20.2)	0.11	33.35 ± 6.8 (17.6–46.7)	0.06
	Non hypertensives (n = 64)	10.94 ± 0.94		13.22 ± 1.74		31.01 ± 5.76	
d	Family history of CAD present# (n = 30)	11.74 ± 0.94 (9.9–13.6)	**< 0.001**	14.78 ± 2.19 (11.1–20.2)	**< 0.001**	35.8 ± 6.21 (19.5–46.7)	**< 0.001**
	No family history of CAD (n = 75)	10.83 ± 0.94		12.94 ± 1.59		30.37 ± 5.62	
e	BMI [kg/m^2]	10.58 ± 0.96	**p < 0.001 (for trend)**	12.69 ± 1.75	**p < 0.001 (for trend)**	28.8 ± 5.68	**p < 0.001 (for trend)**
	18–24.9 (n = 70)						
	25–29.9 (n = 21)	10. 86 ± 0.57		14.01 ± 1.48		34.74 ± 4.38	
	30–34.9 (n = 10)	12.43 ± 0.33		14.27 ± 2.45		33.85 ± 5.93	
	≥ 35 (n = 4)	11.33 ± 1.21		14.83 ± 2.89		35.45 ± 7.01	
f	Dyslipidemia##	11.28 ± 1.15	**0.01**	13.8 ± 2.21	**0.02**	32.8 ± 7.08	0.07
	Present (n = 64)	(9.1–14)		(9.6–20.2)		(17.6–47.5)	
	Absent (n = 41)	10.79 ± 0.72		12.93 ± 1.35		30.55 ± 4.49	

MPV — mean platelet volume; PDW — platelet distribution width; P-LCR — platelet large cell ratio; DM — diabetes mellitus; BMI — body mass index

CBC, fasting and post-meal blood sugar, lipid profile, microalbuminuria, Sr creatinine, eGFR by Cockcroft Gault formula and glycosylated haemoglobin (HbA_{1c}) by standardized HPLC method were performed in all cases. In controls CBC for PVI and fasting, post meal blood sugar and HbA_{1c} and other relevant investigations were done.

Platelet volume indices [5] — the PVI are universally available with routine blood counts by automated

hemograms. Following PVI were studied in cases and controls.

Platelet count: Platelet counts are laboratory measure of concentration of platelets in blood. The standard ranges from 1.5 to 4.5 × 10^6/cu mm.

Mean platelet volume (MPV): This parameter provides a statement on the MPV between the lower discriminator and the upper discriminator. The standard ranges from 8 to 12 fL.

Platelet distribution width (PDW): PDW indicates the platelet distribution width measured at 20% relative height of the total height of the curve. An increased PDW is an indicator for the anisocytosis of platelets. Standard PDW ranges from 9 to 14 fL.

Platelet large cell ratio (P-LCR): P-LCR indicates the percentage of large platelets with a volume > 12 fL and is presented in %. The standard range is 15–35%. An increase may be an indication for platelet aggregates, micro erythrocytes and giant platelets also regenerating large platelets.

Plateletcrit (Pct): Plateletcrit is equivalent to the sum of platelet impulses which are individually detected by means of the impedance measurement principle and thus it is the platelet equivalent to the hematocrit of the RBCs. The standard ranges from 0.22 to 0.24%.

Carotid intima medial thickness: Carotid Doppler was done by single expert sonologist in all cases evaluating both the carotid arteries. Thickening of the intima-media greater than 0.8 mm was considered as abnormal and represented the earliest change of atherosclerosis. Cut-off was based on findings of screening of normal persons and patients with subclinical atherosclerosis prior to this study at our institute by qualified sonologist. Carotid plaques and percentage carotid stenosis were also determined. The far wall of the common carotid artery 1–2 cm proximal of carotid bulb was used for measurement. The leading edge of vascular lumen-intima was selected as the internal measurement site and the leading edge of the media-adventitia as the external limit. The measurement was repeated twice at the same site and then performed in the same way three times at the corresponding opposite site. Mean of CIMT of three sites of a side was taken for calculation of CIMT of that side.

Statistical analysis

Collected data was entered in MS-Excel 2010 and corrected for typographic errors and analyzed using SPSS 16.0 version. Descriptive statistics like proportions, mean and standard deviation was used for continuous variables. For categorical variables, chi-square test was used. To decipher independent association of PVI with CIMT, multiple logistic regression analysis was done with CIMT as the dichotomous outcome variable (dependent variable). Independent T test (unpaired t test) was used to compare between two groups and ANOVA was used to compare the continuous variables among more than two groups. The confidence limit for significance was fixed at 95% level with p-value < 0.05.

Results

It was observed that out of 105 cases and controls, 48 (46%) were males and 57 (54%) were females with a M:F ratio 1:1.2. Most cases (34%) and controls (32%) were in the age group of 51–60 years. In controls, as the age advanced, mean of all platelet indices increased and the difference was statistically significant. The MPV increased from 10.8 ± 0.91 in 32–40 years age group to 11.7 ± 0.82 in 71–80 years age group (p = 0.01). This was not observed in diabetics. This could be due to baseline increase in MPV, PDW, P-LCR in diabetics due to disease itself. Hence the age-related increment did not occur. There was no significant difference in mean MPV, PDW and P-LCR based on gender.

Most patients (59%) had duration of diabetes of more than 5 years, 11.4% cases were newly diagnosed and 11.4% had diabetes of more than 10 years. The mean PVI gradually increased with duration of diabetes up to10 years and were significant for MPV, PDW and P-LCR. Beyond 10 years, PVI were not much altered. This could be explained as number of patients with duration greater than 10 years were much less (11%) and the disease related changes in these platelet indices had already occurred and may have reached a plateau (Table 1).

PVI in T2DM as compared to healthy controls

It was observed that all the platelet indices except platelet count were increased in diabetics as compared to healthy controls. 16% of diabetics had increased MPV, 34% had increased PDW, 26% had increased P-LCR and 76% had increased plateletcrit as compared to 1%, 15%, 23%, 63% controls respectively. Platelet count was within normal range. Mean of MPV, PDW, P-LCR and plateletcrit were also increased in cases as compared to controls, however statistically significant increase was observed only in MPV and P-LCR (Table 2).

Cardiovascular risk factors and PVI

Dyslipidemia (61%) (increased cholesterol and triglycerides and decreased HDL) was the most common cardiovascular risk factor in diabetics followed by hypertension (40%), BMI ≥ 25 kg/m^2 (34%) and family history of IHD (28.6%).

Mean MPV, PDW were significantly increased in patients with hypercholesterolemia, and mean MPV, PDW

Table 2. Platelet volume indices (PVI) in T2DM and non-diabetics

PVI	Cases (T2DM) (n = 105)	Controls (nondiabetics) (n = 105)	p-value
MPV (fL)	11.09 ± 1.02 (8.2–14)	10.28 ± 0.96 (6–12.1)	< 0.001
PDW (fL)	13.46 ± 1.96 (9.4–20.2)	12.85 ± 3.54 (4.2–38.6)	0.12
P-LCR (%)	31.92 ± 6.23 (17.6–47.5)	27.94 ± 5.94 (14.5–39.5)	< 0.001
Platelet count (10^6/cu mm)	2.69 ± 0.55 (1.63–4.26)	2.82 ± 0.69 (1.5–4.57)	0.14
Plateletcrit (%)	0.29 ± 0.05 (0.18–0.47)	0.28 ± 0.07 (0.13–0.5)	0.18

MPV — mean platelet volume; T2DM — type 2 diabetes mellitus; PDW — platelet distribution width; P-LCR — platelet large cell ratio

Table 3. Association of platelet volume indices (PVI) with carotid intima media thickness (CIMT)

PVI Mean + SD (range)	CIMT < 0.8 (n = 49)	CIMT ≥ 0.8 (n = 56)	p-value
MPV (fL)	10.57 ± 0.83 (8.2–12.8)	11.53 ± 0.97 (9.1–14)	**< 0.001**
PDW (fL)	12.53 ± 1.39 (9.4–16.1)	14.28 ± 2.03 (9.6–20.2)	**< 0.001**
P-LCR (%)	28.66 ± 4.78 (19.3–42.5)	34.76 ± 6.06 (17.6–47.5)	**< 0.001**
Platelet count (10^6/cu mm)	2.75 ± 0.60 (1.75–4.26)	2.65 ± 0.51 (1.63–3.52)	0.37
Plateletcrit (%)	0.27 ± 0.06 (0.18–0.47)	0.29 ± 0.054 (0.19–0.4)	0.14

MPV — mean platelet volume; PDW — platelet distribution width; P-LCR — platelet large cell ratio

and P-LCR were significantly increased in patients with hypertriglyceridemia. Mean MPV, PDW and P-LCR were significantly increased in female patients with HDL less than 50 mg/dl as compared to those with normal levels.

PVI like MPV, PDW and P-LCR were significantly increased in cases with BMI ≥ 25 and family history of IHD. There was no significant association of PVI with CV risk factors like hypertension, alcoholism and smoking (Table 1).

Carotid atherosclerosis and platelet volume indices

All diabetics underwent carotid doppler and it was observed that on right side, 44% patients had CIMT < 0.8 mm, 55% had CIMT ≥ 0.8 mm. On left side, 46% had CIMT < 0.8 mm, 53% had CIMT ≥ 0.8 mm. The mean CIMT of both sides were comparable (0.78 ± 0.22 mm on right and 0.79 ± 0.23 mm on left). On right side, 4.7% cases had < 50% stenosis and exactly equal cases had ≥ 50% stenosis, whereas on left, 7.6% cases had < 50% stenosis and no patient had ≥ 50% stenosis. Plaques without significant occlusion were detected on right side in 20.9% and on left side in 8.5% cases. When PVI were correlated with CIMT it was observed that mean MPV, PDW, P-LCR were significantly increased, plateletcrit was marginally increased and mean platelet count was decreased in patients with CIMT ≥ 0.8 (Table 3).

When mean CIMT was plotted against these PVI, it was observed that MPV, PDW and P-LCR had moderate positive linear relationship which was statistically significant. Platelet count had weak negative linear relationship (r = –0.231) and was statistically significant. However, plateletcrit had no relationship with CIMT.

Multiple logistic regression analysis with CIMT as dichotomous dependent outcome variable and significant cardiovascular risk factors as independent predictor variable

MPV was significantly associated and had positive co-relation with age (r = 0.335, p < 0.001), duration of DM (r = 0.437, p < 0.001), BMI (r = 0.268, p = 0.006), cholesterol (r = 0.297, p = 0.002), triglyceride (r = 0.204, p = 0.03), LDL-C (r = 0.222, p = 0.02) and CIMT (r = 0.638, p < 0.001). MPV was significantly but negatively co-related to HDL-C (r = –0.307, p < 0.001). No association existed between MPV and HbA_{1c}.

On MLR analysis, when platelet indices were assessed as independent risk factors after controlling for BMI, dyslipidemia and positive family history of IHD, they were not significant. On univariate analysis, MPV, PDW and P-LCR were significantly associated with increased CIMT. Hence it can be concluded that MPV, PDW and P-LCR are not independent cardiovascular risk factors but may have an effect in association with conventional risk factors, leading to subclinical atherosclerosis as determined by increased CIMT (Figure 1).

Association of PVI with microvascular complications of T2DM

Mean MPV, PDW and P-LCR were significantly higher, and mean platelet count was significantly lower in patients with retinopathy as compared to those without retinopathy. Mean MPV, PDW and P-LCR were signifi-

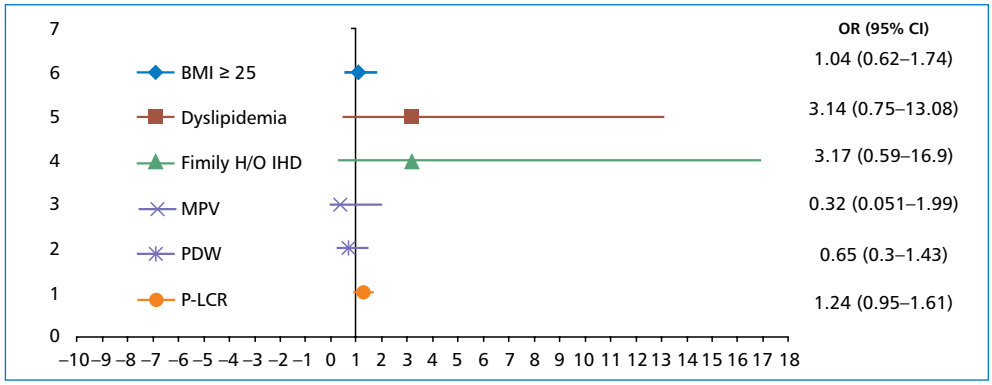

Figure 1. Multiple logistic regressions with CIMT as dichotomous dependent outcome variable. MPV — mean platelet volume; PDW — platelet distribution width; P-LCR — platelet large cell ratio

Table 4. Association of platelet volume indices (PVI) with microvascular complications

Complication	MPV (fL)	p-value	PDW (fL)	p-value	P-LCR (%)	p-value
Retinopathy present (n = 29)	12.09 ± 0.88 (10.3–14)	< 0.001	15.33 ± 1.94 (12–20.2)	< 0.001	37.83 ± 5.09 (25.1–47.5)	< 0.001
Retinopathy absent (n = 76)	10.7 ± 0.79 (8.2–12.5)		12.75 ± 1.44 (9.4–16.1)		29.67 ± 5.12 (17.6–42.5)	
Neuropathy present (n = 27)	11.86 ± 0.88 (9.7–13.6)	< 0.001	14.72 ± 1.74 (11–19.9)	< 0.001	36.26 ± 5.44 (22.8–47.5)	< 0.001
Neuropathy absent (n = 78)	10.82 ± 0.93 (8.2–14)		13.03 ± 1.85 (9.4–20.2)		30.42 ± 5.85 (17.6–46.7)	
Nephropathy present (n = 55)	11.54 ± 1.03 (9.2–14)	< 0.001	14.33 ± 2.03 (11–20.2)	< 0.001	34.7 ± 6.27 (22.8–47.5)	< 0.001
Nephropathy absent (n = 50)	10.58 ± 0.75 (8.2–12.4)		12.51 ± 1.36 (9.4–14.8)		28.8 ± 4.67 (17.6–38.2)	

MPV — mean platelet volume; PDW — platelet distribution width; P-LCR — platelet large cell ratio

cantly higher, and mean platelet count was lower but non-significantly in patients with neuropathy as compared to those without neuropathy. Mean MPV, PDW and P-LCR were significantly higher, and mean platelet count was significantly lower in patients with nephropathy as compared to those without nephropathy (Table 4).

Discussion

Diabetes is considered as a 'prothrombotic' state. Platelet activation is an important factor, the determinants of which are platelet volume indices i.e. mean plate volume (MPV), platelet distribution width (PDW) and platelet large cell ratio (P-LCR). When we compared the PVI in T2DM with healthy controls we observed that MPV, P-LCR were significantly increased in diabetics. PDW and plateletcrit were increased but statistically insignificant and platelet counts were unaltered. In diabetes, there is increased thrombopoiesis and circulation of large, young and activated platelets which undergo more frequent episodes of release of granules, accelerated sequestration in the circulation and have reduced survival, thus platelet count is decreased. As plateletcrit is the volume occupied by platelets in the blood as a percentage, and directly related to platelet count, both the indices decrease in diabetics. However, no such decrease in plateletcrit was seen in our study. Factors that contribute directly to greater platelet reactivity in diabetics include metabolic abnormalities such as hyperglycemia and hyperlipidemia, both insulin resistance (relative insulin deficiency) and absolute insulin deficiency, as well as associated conditions such as oxidative stress, inflammation, and endothelial dysfunction [6].

Many studies have reported increase in MPV in diabetics as compared to non-diabetics. Our results were in accordance to studies done by Kodiatte et al. [7], Papanas et al. [8], and Alhadas et al. [9]. Hekimsoy et al. [10] also reported increased MPV in diabetics but

reported low platelet counts in diabetics. We did not observe any alteration in platelet counts in diabetics. MPV is an indicator of the average size and activity of platelets. Larger platelets are younger, more reactive and aggregable. Hence, they contain denser granules, secrete more serotonin and β-thromboglobulin, and produce more thromboxane A2 than smaller platelets. MPV reflects the state of thrombogenesis. There might be small bleeds due to the rupture of atherothrombotic plaques leading to increased platelet recruitment, hyper reactivity, and bone marrow stimulation. Thus, high MPV is emerging as a new risk factor for the vascular complications of DM of which atherothrombosis plays a major role [7]. Many researchers have observed an association of increased MPV with cardiovascular events like myocardial infarction and acute coronary syndrome. We did find an increased PDW in diabetics, but the difference was statistically insignificant. Contrary to this Jindal et al. [11] observed that PDW was significantly higher in diabetics. It could be due to larger platelets being hyper-reactive produce more prothrombotic factors. Platelet activation causes changes in platelet morphology and formation of pseudopodia. The enlarged platelets with lots of pseudopodia differ in the size, possibly affecting the platelet distribution. Our study also observed statistically significant increase in P-LCR which implied that platelet size was larger in diabetics. This finding has been corroborated by many studies.

Studies have shown that platelet count and size might be gender and age dependent. Advanced age was directly associated with increased MPV as concluded in a study by Lippie et al. [12], where they attributed this increase in platelet volume to other cardiovascular risk factors like hypertension, dyslipidemia etc. that occur with advancing age. We observed that MPV, PDW and P-LCR also increased significantly with age in controls, but not in diabetics. There was no significant association of platelet count and plateletcrit with age.

It was observed that platelet count and plateletcrit was increased significantly in females as compared to males. It was in accordance with a study by Bain et al. [13]. The difference can possibly be ascribed to the different hormonal profiles amongst the gender or the effect of menstrual cycle on the haemostatic mechanism as thrombocytosis is associated with bleeding. There were no significant differences among the genders with respect to MPV, PDW or P-LCR.

When conventional cardiovascular risk factors were studied, it was observed that, dyslipidemia (61%) was the most common risk factor in diabetics followed by hypertension (40%), BMI $\geq$ 25 kg/m^2 (34%) and family history of CAD (28.6%). In controls, dyslipidemia (28%) was the most common risk factor followed by hypertension (27%), smoking (19%), and family history of CAD (17%). PVI were significantly higher in diabetics with dyslipidemia (MPV and PDW), family history of CAD (MPV, PDW and P-LCR) and BMI $\geq$ 25 kg/m^2 (MPV, PDW and P-LCR). PVI were high in patients with hypertension and smoking but statistically not significant. Nechita et al. [14] also observed that patients with cardiovascular risk factors, especially complex ones like the metabolic syndrome had an increased MPV, as did the patients with unstable angina whether associated with the risk factors. They implied that MPV could be used to differentiate unstable angina from non-cardiac chest pain. In a study by Swaminathan et al. [15] MPV was found to be higher in subjects with type 2 diabetes and significantly increased in diabetics with poor glycemic control and having a longer duration of diabetes. In contrast to most studies we did not find a significant association between PVI and glycemic control [16].

PVI and carotid intima media thickness

Carotid doppler was performed in all diabetics and patients were divided in 2 groups based on CIMT; CIMT $<$ 0.8 and CIMT $\geq$ 0.8 mm. PVI were compared between 2 groups. It was observed that mean MPV, PDW, P-LCR were increased significantly in patients with CIMT $\geq$ 0.8. Mean platelet count was decreased and plateletcrit was increased but statistically insignificant.

In diabetic patients, hyperglycemia impairs vascular endothelial cells and NO production which contributes to the formation of atherosclerosis by mediating leukocyte activation and adhesion to the endothelium via platelet P-selection [17]. As the platelets participate in the formation of atherosclerotic plaque, they are consumed and platelet count may decrease with or after the development of atherosclerosis. Our study revealed that platelet count has weak negative linear relationship with CIMT ($r = -0.231$) which was statistically significant. Also, other indices like MPV, PDW and P-LCR had moderate positive linear relationship with CIMT which was statistically significant. However, plateletcrit had no relationship with CIMT.

Many efforts have focused on the elucidation of common pathophysiological mechanisms in obesity, type 2 diabetes and atherosclerosis, supporting the role played by inflammation. A feature of inflammatory activity is the increase in circulating plasma of acute-phase proteins produced by the liver such as C-reactive protein (CRP) and fibrinogen. Fibrinogen levels have been shown to be associated with enhanced platelet aggregation and smooth muscle cell proliferation. Furthermore, there is a strong association of fibrino-

gen with blood viscosity and thrombus formation and circulating levels of fibrinogen have been known to have a strong and consistent relationship with CAD [18]. However, in our study we did not estimate the hsC-reactive protein or fibrinogen levels, and this is a limitation of this study which may have overemphasized the role of platelet indices.

According to Wan et al. [19] MPV had a high positive correlation with IMT ($p < 0.001$). Adam et al. [20] in their correlation analysis showed a positive association between PDW and the degree of carotid stenosis. However, they did not find a significant correlation between carotid stenosis and MPV. According to study by Yilmaz et al. [21] the calcified plaque group was compared with the non-calcified plaque group in terms of MPV and PDW. The findings of their study indicated that the non-calcified plaques that cause intermediate carotid artery stenosis are associated with a significantly higher MPV value compared to the calcified plaques. There was no difference between the two groups with respect to PDW values. However, study by Kim et al. [22] found that MPV was strongly associated with the severity of glycemic control but not significantly associated with the early and late stages of atherosclerotic vascular changes in type 2 diabetes mellitus patients. They concluded that MPV is not a reliable marker for subclinical atherosclerosis in a diabetic population. This was possibly confounded by the close association of MPV with poor glycemic control.

Thoracic aortic intima-media thickness (IMT) was reported as an earlier marker of preclinical atherosclerosis than carotid IMT. Yüksel Kalkan et al. [23] reported that MPV is independently related to the extent of subclinical thoracic aortic atherosclerosis .Thus increases in MPV may be a crucial biochemical marker for initial atherosclerosis.

On multiple logistic regression analysis with CIMT as dichotomous dependent outcome variable and significant cardiovascular risk factors as independent variables, it was observed that MPV, PDW and P-LCR were not independent risk factors for cardiovascular disease, but had an effect in association with conventional risk factors, leading to subclinical atherosclerosis as determined by increased CIMT, although on univariate analysis, these PVI were significantly associated with increased CIMT.

If vascular damage was only due to increased number of large and reactive platelets, then the rate of damage would have been constant for the duration of disease and independent of diabetic control. This clearly shows that platelet reactivity alone cannot explain the progression of vascular complications in DM since there are other vascular risk factors that may be influenced by degree of control of diabetes. Platelet number and reactivity along with cardiovascular co-morbidities such as hypertension, albuminuria, obesity, cigarette smoking, and dyslipidemia also contribute to progression of diabetes and its effect on PVI. Thus, it shows that there are other factors which may account for the thrombotic potential of diabetics.

According to Alvitigala et al. [24] and Majumdar et al. [25] PVI have the potential to be used as a preliminary test to identify high-risk patients for myocardial infarction along with other supportive clinical investigations.

PVI and microvascular complications

Studies have found an association between increased MPV and CAD but very few studies have explored the relation between PVI and all the microvascular complications. In our study, diabetic nephropathy (82%) was the most common microvascular complication, followed by retinopathy (46%), and neuropathy (46%). MPV, PDW and P-LCR were significantly higher in diabetics with microvascular complications. Platelet count was significantly increased in retinopathy and nephropathy but insignificantly in neuropathy. Papanas et al. [8] found that MPV was higher in patients with microvascular complications. Ates et al. [26] proved an association between degree of retinopathy and mean MPV. Buch et al. [27] and Alhadas et al. [9] concluded that MPV and PDW were significantly associated with microvascular complications. Jindal et al. [11], found that only PDW was significantly higher in diabetics with complications. This suggests a role of the enhanced platelet activity in the pathogenesis of microvascular complications.

Thus, PVI namely MPV, PDW, P-LCR are increased in diabetics and have a positive association with increased CIMT. They also correlate with all 3 microvascular complications of diabetes. They are not independent predictors but may be useful marker of carotid atherosclerosis in association with other cardiovascular risk factors.

Limitations of the study

Time bound study with relatively small sample size. Many confounding factors for poor glycemic control and increased complications in our subset of patients may have overestimated the association of platelet volume indices with microvascular complications. Also, we could not estimate hs CRP and fibrinogen levels which are also surrogate markers of inflammation associated with atherosclerosis.

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Conflict of interest

The authors declare to have no conflict of interest.

Contributors: *RW conceived the study, reviewed the literature, acquired the data, analyzed it and drafted the article. RSK formulated the study design, helped with informed consent of the patients and investigations, and edited the article. PPJ helped refining the study design and helped in preparation of final version of the article.*

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Impact of smoking and nicotine addiction on HbA_{1c} levels and diabetic microvascular complications

ABSTRACT

Introduction. In this study we aimed to determine whether a difference in complications between smokers and non-smokers exists in type 2 diabetes mellitus (T2DM) and to evaluate if there is a correlation between microvascular complications and Fagerström test score.

Material and methods. Patients with T2DM who attended the family medicine outpatient clinics were enrolled in the study. Smokers and non-smokers were compared according to their metabolic outcomes and presence of microvasculer complications. The level of smoking addiction was determined by Fagerström Test for Nicotine Dependence.

Results. Fasting blood glucose (FBG), low-density lipoprotein, systolic and diastolic blood pressures were found to be higher in smokers. The presence of neuropathy was significantly higher in smokers. The presence of retinopathy rate increased with increasing level of smoking addiction. The nicotine dependence test score were found to be positively correlated with HbA_{1c} and FBG levels whereas, negatively correlated with body mass index among smokers.

Conclusions. Assessing the cigarette smoking status of diabetic patients at the initial clinic visit and indicating the importance of smoking cessation should be the essential part of diabetes follow up program. (Clin Diabetol 2020; 9; 2: 112–117)

Key words: smoking, diabetes mellitus, microvascular complications, fagerstrom, HbA_{1c}

Introduction

Type 2 diabetes mellitus (T2DM) is a major health and socioeconomic problem for the community. According to the World Health Organisation's (WHO) data 422 million adults were living with DM in 2014 [1]. The number of adults with DM reached 451 million in 2017 and keeps increasing [2]. It is predicted that 642 million people will have DM and 481 million people will have impaired glucose tolerance by the year 2040 [3]. DM directly caused 1.6 million deaths in 2016 [4].

More than 1.1 billion people worldwide aged 15 years or older smoked tobacco in 2016 [4]. Smoking is the utmost known modifiable risk factor for many chronic diseases such as cardiovascular disease, chronic obstructive lung disease, and T2DM. Association between smoking and T2DM was investigated in previous studies [5]. According to a recent study, genetic polymorphisms in the nicotinic acetylcholine receptor genes may contribute to the association between smoking and T2DM [6]. Smoking is an independent risk factor for T2DM and both active smoking and exposure to passive smoke increase the risk of T2DM [7]. Compared with non-smokers with no exposure to passive smoke, there is an increased risk of T2DM among nonsmoker exposure with passive smoke [8]. Furthermore, smoking is found to be a risk factor for chronic complications of T2DM such as progression of diabetic nephropathy, peripheral polyneuropathy and diabetic retinopathy [9–13].

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This study aims to determine whether a difference in microvascular complications between diabetic patients who are smokers and non-smokers exists and to evaluate if there is a correlation between microvascular complications and smoking addiction.

Methods

Study population

T2DM patients who attended the family medicine outpatient clinics of a referral hospital were enrolled consecutively in the study. Patients with gestational diabetes, secondary diabetes; retinopathy, nephropathy or neuropathy not caused by diabetes; oncologic, inflammatory, immunologic or neuropsychiatric disease, ex-smokers and patients younger than 18 were excluded. A questionnaire regarding medical history and lifestyle factors was used during the clinic examinations.

Data collection and measurements

A structured checklist was used to record patients' demographics and laboratory data. We measured height to the nearest centimeter, weight to the nearest 0.5 kg, and systolic blood pressure (SBP) and diastolic blood pressure (DBP) to the nearest mm Hg. Blood pressure was measured with a standard mercury sphygmomanometer while the patient was sitting after resting for 10 minutes and a mean of 3 readings was recorded. The subjects had their body weight (kg) assessed in the morning after overnight fasting. Bodyweight was measured using digital scales with the subjects only wearing underwear. BMI was calculated as weight (kg) divided by height (m) squared.

Smokers' severity of dependence was determined with The Fagerström Nicotine Dependence Test. In accordance with test scores, subjects were considered as minimally dependent (4 points or less), moderately dependent (5 to 7 points) and highly dependent (8 to 10 points).

Patients were examined by an ophthalmologist with an ophthalmoscope to determine retinopathy. Microaneurysm, retinal hard exudates, retinal edema, and retinal new vessels are evaluated positively for retinopathy. Blood creatinine levels, glomerular filtration rate (GFR) and elevated albuminuria of > 300 mg/24 h with concurrent presence of diabetic retinopathy and absence of signs of other forms of renal disease were used to evaluate nephropathy [14]. GFR is calculated with the CKD-EPI equation: $GFR = 141 \times \min(SCr/\kappa, 1)^{\alpha} \times \max(SCr/\kappa, 1)^{-1.209} \times 0.993^{age} \times 1.018$ (for women) (SCr is serum creatinine [mg/dL], κ is 0.7 for females and 0.9 for males, α is –0.329 for females and –0.411 for males, min indicates the minimum of SCr/κ or 1, and max indicates the maximum of SCr/κ or 1). Patients with GFR < 60 ml/min, > 300 mg albumin levels in 24-hour urine sample were diagnosed with nephropathy.

Patients were assessed for neuropathy using their medical history and examination. Symptoms especially worsened or occurring mostly during the night such as pain, dysesthesias (unpleasant sensations of burning and tingling), numbness and loss of protective sensation were considered as neuropathy symptoms. Comprehensive physical examination was performed to examine neuropathy include muscle power and tone examination, vibration perception examination with 120–200 Hz diapason, temperature and pinprick sensation examination, proprioception examination, 10-g monofilament to assess light-touch perception and ankle reflexes examinations are done to detect neuropathy [15]. Any abnormal examination result was evaluated as neuropathy. Electroneurography was not performed.

Patients' exercise and diabetes educational status were assessed. Patients who exercised at least 30 minutes per day and 3 to 7 days of the week were assessed as performing exercise regularly. Patients who had already been informed by a health professional including general practitioner or family physician about the course of T2DM, its complications and treatment options were considered educated. ADVIA 2400 Chemistry Systems/SIEMENS device was used to detect fasting plasma glucose (FBG), postprandial plasma glucose (PBG), total cholesterol (TC), low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglyceride (TG), protein levels in 24-hour urine samples. HbA_{1c} levels are detected by (HPLC) Hb9210/PREMIER device which used boronate affinity technology.

Statistical analyses

We used IBM SPSS version 22 statistics for statistical analysis. Data's compatibility with normal distribution was determined by the Kolmogorov-Smirnov test. Comparisons between groups were made using Student's t test (for continuous variables) and the Chi-square test (for categorical variables). All values are expressed as the mean ± SD or number (%). We assumed $p < 0.05$ indicated significant differences.

Results

A total of 210 patients with T2DM (64.3% men) were enrolled into the study. The mean age of the patients was 49.7 ± 14.5, mean BMI was 26.2 ± 4.4 kg/m^2 and the mean T2DM duration was 8.3 ± 5.9 years. A total of 70 (33.3%) participants were active

Table 1. Comparison of demographical and clinical features of smokers and non-smokers

	Non-smoker	Smoker	p
Gender			
Women, n (%)	90 (64.3%)	45 (64.3%)	1.000
Men, n (%)	50 (35.7%)	25 (35.7%)	
Age (year) (MSD)	49.8 ± 14.7	49.4 ± 14.1	0.848
BMI [kg/m^2] (MSD)	26.1 ± 3.7	26.5 ± 5.4	0.507
Diabetes duration (year) (MSD)	8.1 ± 5.90	8.5 ± 6	0.679
Exercise			
No, n (%)	133 (95%)	61 (87.1%)	**0.043**
Yes, n (%)	7 (5%)	9 (12.9%)	
Education			
< High school, n (%)	63 (45%)	36 (51.4%)	**0.038**
≥ High school, n (%)	77 (55%)	34 (48.6%)	
Diabetes education			
No, n (%)	49 (35%)	16 (22.9%)	0.073
Yes, n (%)	91 (65%)	54 (77.1%)	
Systolic blood pressure (MSD)	120.3 ± 12.9	124.5 ± 15.3	**0.050**
Diastolic blood pressure (MSD)	73.5 ± 10	79.5 ± 10.2	**0.001**
Fasting blood glucose [mg/dL] (MSD)	163.1 ± 59.1	193.5 ± 84.8	**0.008**
Postprandial blood glucose [mg/dL] (MSD)	252.8 ± 96.5	281.2 ± 114.3	0.061
HbA_{1c} (%) (MSD)	8.8 ± 2	9.3 ± 2.3	0.133
Total cholesterol [mg/dL] (MSD)	179.9 ± 46.3	188.3 ± 46.7	0.216
LDL [mg/dL] (MSD)	102.2 ± 36.2	112.7 ± 33.4	**0.042**
HDL [mg/dL] (MSD)	41 ± 11.5	42.6 ± 13	0.382
TG [mg/dL] (MSD)	184.7 ± 134.4	171 ± 93	0.443

MSD — mean standard deviation; BMI — body mass ındex; HbA_{1c} — glycated hemoglobin; LDL — low-density lipoprotein; TG — triglyceride; HDL — high--density lipoprotein

smokers. Their mean smoking duration was 26.5 ± 17.5 pack-year. Comparison of demographical and clinical features of smokers and non- smokers are presented in Table 1. SBP (p = 0.05) and DBP (p = 0.001), FBG (p = 0.008) and LDL (p = 0.042) were found to be higher in smokers, respectively. Although the mean HbA_{1c} was found to be higher in smokers, it was not statistically significant. There were no significant differences between smokers and non-smokers in terms of their antidiabetic medications (Table 2).

The presence of neuropathy was significantly higher in smokers (Table 3). The presence of retinopathy rates increased with an increasing level of smoking addiction (p = 0.015) (Table 4). The nicotine dependence test score was found to be positively correlated with HbA_{1c} and FBG levels whereas, it negatively correlated with body mass index among smokers (p = 0,042) (Table 5).

Table 2. Antidiabetic drug usages among smoker and non-smoker patients

	Non-smokers n (%)	Smokers n (%)	p
Anti-diabetic medications			
Oral antidiabetic drugs	52 (37.1)	27 (38.6)	0.920
Insulin	36 (25.7)	19 (27.1)	
Insulin and oral antidiabetic drugs	52 (37.1)	24 (34.3)	

Discussion

Association between smoking and impaired glucose control was examined in many previous reports [16–18]. FBG levels were found to be higher in smokers compared to non-smokers in the present study.

Table 3. Comparison of microvascular complications of smokers and non-smokers

	Non-smokers n (%)	Smokers n (%)	p
Retinopathy			
Negative	99 (70.7)	48 (68.6)	0.749
Positive	41 (29.3)	22 (31.4)	
Nephropathy			
Negative	86 (61.4)	36 (51.4)	0.166
Positive	54 (38.6)	34 (48.6)	
Neuropathy			
Negative	113 (80.7)	43 (61.4)	**0.003**
Positive	27 (19.3)	27 (38.6)	

Table 4. Comparison of microvascular complications between smoking addiction levels

	Nicotine dependence			p
	Low n (%)	Medium n (%)	High n (%)	
Retinopathy				
Negative	21 (91.3)	14 (60.9)	13 (54.2)	**0.015**
Positive	2 (8.7)	9 (39.1)	11 (45.8)	
Nephropathy				
Negative	15 (65.2)	10 (43.5)	11 (45.8)	0.268
Positive	8 (34.8)	13 (56.5)	13 (54.2)	
Neuropathy				
Negative	17 (73.9)	15 (65.2)	11 (45.8)	0.128
Positive	6 (26.1)	8 (34.8)	13 (54.2)	

Table 5. Correlation of Fagerstrom Nicotine Depence Test score with clinical and laboratory features

	r	p
Age	0.082	0.5
BMI	–0.244	0.042
Fasting blood glucose	0.247	0.039
Post prandial blood glucose	0.211	0.080
HbA_{1c}	0.244	0.042
Total cholesterol	–0.40	0.741
LDL	0.078	0.520
TG	0.043	0.724
HDL	–0.183	0.128

BMI — body mass ındex; HbA_{1c} — glycated hemoglobin; LDL — low-density lipoprotein; Tg — triglyceride; HDL — high-density lipoprotein

In addition, PBG levels were higher in smokers whereas no statistically significant difference was found. Furthermore, HbA_{1c} levels were higher in smokers. This finding is inconsistent with other previous reports [16, 19]. All those increases could be partly explained by impaired glucose metabolism and insulin secretion due to smoking [20, 21].

It has been shown that achieving and maintaining glucose control is more difficult in smokers [16], hence microvascular complications can be more significant among diabetics who smoke. Smoking is one of the most important modifiable risk factors for the progression of microvascular complications and blood glucose dysregulation in diabetic patients. Smoking increases T2DM incidence and worsens T2DM complications. Although the mean HbA_{1c} was significantly higher in current smokers, the difference between smokers and never-smokers in FBG and PBG was controversial in previous studies [16].

In our study, the prevalence of cigarette smoking among diabetic patients was found to be 33.3%. This ratio is comparable with smoking ratio among the Turks. According to WHO, one-third of Turkish adults smoke cigarettes [22].

Smoking immediately increases SBP and DBP due to an increased in sympathetic nervous system activities and released of epinephrine, norepinephrine and vasopressin hormones [23, 24]. However, smoking's long term effect on blood pressure is not revealed [25]. Previous studies with older smokers found a larger difference in SBP compared to studies with younger smokers among T2DM patients [18]. It is an important outcome, considering that most of the smokers with T2DM were likely to be older. In this study, we found statistically significant differences in both DBP and SBP between smokers and non-smokers.

Previous studies indicated that smoking exerts a negative effect on lipid profiles [18, 26]. We found that LDL was significantly higher in smokers compared with non-smokers, as in previous reports [18]. Whereas, in HDL and TG statistically significant difference was not determined between smokers and non-smokers in our study.

One-third of T2DM patients have been diagnosed with microvascular complications at the time of diabetes diagnosis. However, the association between smoking and the development of microvascular complications has not been fully elucidated [27].

Retinopathy takes years to develop but eventually appears in nearly all diabetic patients. Pathogenesis of diabetic retinopathy related smoking has not been clearly established, impaired retinal microcirculation may be implicated [28]. Previous studies of the association between smoking and diabetic retinopathy had different results [28–30]. In our study, the presence of retinopathy rate was positively associated with the

Fagerström Test for Nicotine Dependence score. In addition, smokers have a higher rate of retinopathy than non-smokers, however, a statistically significant difference was not found.

A previous meta-analysis indicated that smoking is associated with the prevalence and incidence of diabetic neuropathy [12]. This study revealed that neuropathy has a significant difference between smokers and non-smokers, as in previous reports. Previous studies assessed that smoking is associated with diabetic nephropathy [9, 31]. Moreover, the smoking amount showed a dose-response relationship with albuminuria [31–33]. We also found that smokers have a higher rate of nephropathy comparing to non-smokers. However, the result was not statistically significant.

We acknowledge that our study has several limitations. First of all, although our study was conducted in one of the largest hospitals in Ankara which admits average of 6000 patients per day, it is a mono-centered study and the number of subjects in this study was not large. Therefore the generalisability to other population groups is uncertain. Second, we enrolled only T2DM patients. Consequently, we did not have chance to evaluate our findings in different diabetic groups. Third, this is a cross-sectional analysis, precluding inference of causality to the observed associations. The present findings should be replicated in a future study with a higher number of subjects, conducting a prospective assessment of each parameter.

Conclusions

In conclusion; our results indicate that smokers with T2DM are more likely to have diabetic neuropathy complication compared to non-smokers. In addition, the presence of retinopathy rate increased with increasing level of smoking addiction. Furthermore, the nicotine dependence test score were found to be positively correlated with HbA_{1c} and FBG levels whereas, negatively correlated with body mass index among smokers. Those findings reaffirm the need for clinical research to test tailored smoking cessation interventions for people with T2DM.

Smoking cessation might cause a reduction in microvascular complications. Unfortunately, there are limited data available to inform smoking cessation in people with diabetes. Therefore, it is thus of paramount importance to design intensive and innovative interventions to quit smoking in T2DM. The potential benefits of giving up smoking in those patients should be tested and evaluated in future studies.

All diabetes care professionals should be aware of the addictive and harmful effects of smoking.

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Sugary beverages consumption and latent autoimmune diabetes in adults: systematic review and meta-analysis

ABSTRACT

Introduction. Sugary beverages consumption (SBC) has amplified globally. SBC is associated with and leads to obesity and chronic diseases, nonetheless the role of SBC in development of autoimmune disorders such as latent autoimmune diabetes in adults (LADA) has not been addressed adequately among the different ethnic groups. We conducted this meta-analysis to compare the random effect of SBC intake on the risk of development of LADA.

Methods. We scrutinized the MEDLINE database up until January 2019 for articles addressing the association between sugary beverages, coffee consumption and LADA. We found 6 studies all of them addressed the LADA. We have included them in the meta-analysis and compared the random effect of SBC from the uppermost to the lowermost quantiles parallel to the risk of LADA.

Results. According to the research conducted, and data extracted, which involved 15027 contributors and 1862 patients with LADA, the participants in the uppermost quantile of SBC intake (used 1–2 servings per day in most cases) were at risk of developing LADA more than those in the lowermost quantile (≤ 1 serving per month) (odds ratio [OR] 1.37 [95% CI 1.23–1.52]).

Conclusion. According to the meta-analysis results excessive SBC intake may increase the risk of development of latent autoimmune diabetes in adults. However, no definite conclusions could be drawn due to heterogeneous data from low quality researches and the analysis was based on observational and case-control studies only. (Clin Diabetol 2020; 9; 2: 118–127)

Key words: sugary beverages consumption, latent autoimmune diabetes in adults, systematic review, meta-analysis

Introduction and background

In almost 50 years sugary beverages consumption (SBC) has increased at an alarming rate worldwide. For instance, in the United States, from 1970 to 2006 SBC per each individual reised from 64.4 to 141.7 kcal/ /day, forming double or twice the increase [1]. Similar results have been revealed in Mexico, where presently more than 12% of total calorie intake was represented by SBC [2]. The rapid and dramatic increase of SBC in several developing republics where SBC has increased concurrently in relation with increasing rates of growth and urbanization. In the 2007 annual report, the Coca-Cola company shows that, the amount of SBC sold in India and China increased by 14% and 18% respectively in one year, indicative of the considerable upsurges in trade at the national level [3]. For clarification sugary beverages include carbonated sodas, energy drinks, sport drinks, juice drinks, sweetened tea, iced tea, fruit drinks, and vitamin drinks. Recent research has been

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updated to include sweetened coffee and alcoholic beverages as sugary sweetened beverages (SSBs) [4]. Sugary beverages are currently the main source of supplementary additional sugars in a typical American diet. They contain multiple sweeteners such as corn fructose, sucrose, fruit juice extracts, all of which have the same basic analogous metabolic drawbacks [5].

On the other hand, a drink that is a 100% pure and natural fruit juice and not mixed with extra sugars is not considered a sugary sweetened beverage. progressively, teams of researchers and institutes are calling for maximum reductions in SBC [5, 6]. Results from significant prospective researches in epidemiology have demonstrated a reliable positive correlation between SBC and obesity among children and adults [7]. Furthermore evolving proof also proposes that habitual SBC is correlated with a higher risk of developing diabetes and other metabolic disorders [8]. SBC has been proven to be the cause of obesity due to their more added sugar and imperfect recompense for total calorie intake [7].

Due to the high amount of fast absorbable sugars such as fructose corn syrup and sucrose, in combination with the large amounts consumed, SBC might increase the risk of diabetes through obesity and by raising the dietary glycemic index, and insulin resistance, which contributes to β-cell dysfunction [9]. An increase in metabolic impacts of SBC may also cause elevated blood pressure and the buildup of visceral fatty tissue and ectopic adiposities due to high liver de novo lipogenesis [10], which will in turn lead to the development of more triglycerides, LDL and decrease the level of HDL. The correlation between SBC and LADA is less clear [11, 12], but current research proposes that SBC may elevate the risk of diabetes in hereditarily predisposed subjects [11]. Conceivable mechanisms for SBC participation in autoimmune pathogenesis involve prompted beta cell apoptosis [13], perhaps due to prompted oxidative stress, high glucose levels [14, 15] or an overwhelmed beta cell, probably because it is more visible and exposed to the body's immunity [16]. LADA is a form of diabetes combining the features of both of type 1 and type 2 diabetes. Besides the involvement of autoimmune indicators such as anti-glutamic acid decarboxylase antibodies (GADA) with LADA. The most common biochemical marker in LADA is a mild or moderate insulin resistance [17]. Therefore, it is likely that SBC may impact the risk of LADA by the pathogenesis related to autoimmune disorders or insulin resistance, but then again this is still unclear. 9% of all cases diagnosed as adult-onset diabetes was recognized as LADA [18], which is considered a mixture of different elements from different types of diabetes. After review of the available literature, we conducted a systematic review and a meta-analysis to observe the relationship between SBC and the risk of development of LADA.

Research methodology and design

Search of literature

Guidelines of the PRISMA 2009 Statement have been adopted — step by step as we conducted our meta-analysis [19]. Pertinent, applicable and multi-ethnic researches written in the English were recognized and acknowledged by an in-depth and meticulous probing of the following databases: MEDLINE electronic database; Cochrane Library; PsycINFO — American Psychological Association; Embase; CAB Abstracts; Web of Science by Clarivate Analytics (formerly known as ISI Web of Knowledge); CINAHL Database, BIOSIS (King Saud University Medical City Library of Medicine, Salah, MD) for studies from 1983 to January 2019, which included SBC and sugar-sweetened beverage consumption, such as: (soft drinks, soda, carbonated drinks, sweetened coffee, iced tea, alcoholic beverages, fruit drinks, squashes, sports drinks, soda-pop, cordials, energy drinks, punch, vitamin water drinks and sugary lemonade) and the risk of LADA. We searched for keywords including those mentioned above as well as those combined with "auto antibodies", "autoimmune disorders of β-cells of the pancreas", "latent diabetes", "latent autoimmune diabetes in adults", and "LADA." We used this method as extensively as possible as our primary means of exploration and in the next successive medical subheading (MESH) terms examination. Every relevant article that was found, we followed its references, searching for any hints of another thread. We searched for references and cross-references that would possibly guide us to other references. We searched not only for articles published in journals, but also those in the press, books, magazines, newspapers, websites, documentary films, dissertations, congressional publications, international organizational reports, and even editorials by deploying the (Citation Machine®) as a means of accomplishing our purpose and achieving our objective.

Due to the high possibility for confusing and converse causation, we have excluded cross-sectional researches. We have also excluded short-term trials as they were unable to address the long-term relationship that we are exploring. However these short-term studies do provide significant intuition about the possible causal biological mechanisms and thus has helped further our understanding of the causality in some capacity.

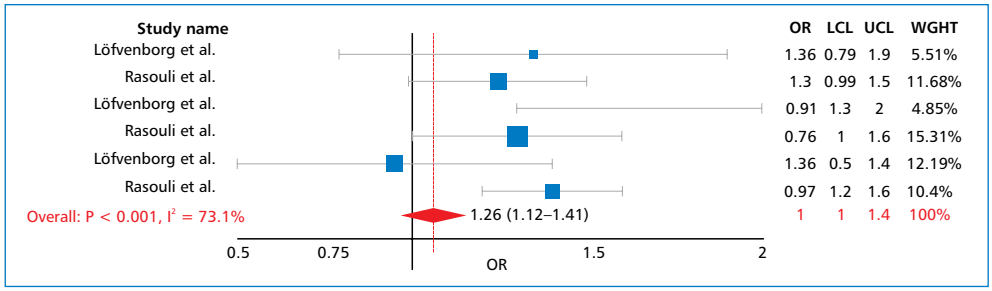

Figure 1A. Forest plot shows researches evaluating SBC and risk of LADA, comparing extreme quantiles of intake, random effects estimate

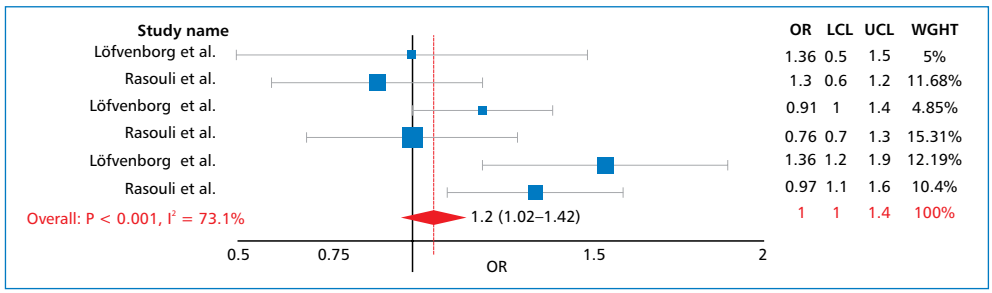

Figure 1B. Forest plot shows researches assessing SBC and type 2 diabetes mellitus comparing extreme quantiles of intake, random effects estimate

Inclusion criteria and extracting data

In our meta-analysis we included population based observational epidemiological studies as inclusion criteria. Criteria for inclusion comprised the end points of LADA, associated measure of variance (standard error or confidence interval) and relative risk as well as measures of SBC and potential mediators' adjustment. After we applied these criteria, our collected works selected eight identified articles out of 148 relevant references. Those 148 references were derived from 7534 citations (Figure 1). Each of the eight studies hit our target precisely [20–27]. Two of these studies have been excluded as one of them was review of literature [27] and the other was a master's thesis which was published as a paper later on, and selected from our target group [24].

The remaining six studies [20–27], all of which were held in Sweden, and written in English and those were two main weak points and mentioned as limitations. Only one study was done in the form of three cross sectional surveys on data from Nord-Trøndelag Health survey (HUNT) in 2012 [23]. The others were performed in the form of population based case-control studies, published in 2016–2019, apart from the two studies which were published in 2014 [21, 22]. All of the studies measure the association between SBC *versus* LADA, using age, sex, BMI, family history, total calorie intake, smoking, and education as potential mediators. Only two addressed the genetic susceptibility as an independent variable [20, 25]. Estimated adjusted odds ratio (OR) of diabetes were entailed in relation to SBC. Standard errors and coefficients of variation were attained from Rasouli et al. [21] and Löfvenborg et al. [22] through subsequent communications. Two of the team members independently extracted the data. No variances were noticed in the extracted data to provide estimation of the effect, comparing skewed or drastic quantiles of SBC. All studies have defined one serving as 200 ml however there are some notable variations in estimation of the serving size including Löfvenborg et al. [20] in which the maximum level of SBC was 200 ml servings per day and lowest amount of intake was 200 ml servings per week. With Rasouli et al. [21], the average intake of alcoholic beverages was 12 grams per day, while the maximum amount served was 25 grams per day. Löfvenborg et al. [22] established that the minimum amount of sweetened coffee per day was < 2 cups while highest amount reached up to > 6 cups. The minimum intake of alcohol per day with Rasouli et al. [23] was < 1 time and > 10 times in the highest category of intake. Löfvenborg et al. [24] classified the amount of servings of soft drinks and sodas as (< 1, 1–2 and > 2 servings per day). Finally the least amount of coffee served with Rasouli et al. [25] was < 1 cup per day and the highest was > 4 cups.

Patient and public involvement statement

Our current study design was conducted in the form of meta-analysis and systematic research on already published study articles, so no patient involvement was documented, and the used materials was only published data.

Limitations of the study

The first limitation and point of weakness is that we conducted this meta-analysis and there was heterogeneity of results. All studies had different populations, different designs and outcomes. However all of them addressed the same topic and the same research question. Available literature regarding this topic is very scarce, in addition to heterogeneous data from low quality studies depending on retrospective, observational or case-control designs only. This in turn reflected negatively on the level of evidence and conclusion. Moreover, there were no prospective or long term experimental, interventional or randomized studies, with sufficient follow-up period, to demonstrate the potential relationship between sugary beverage consumption and LADA, and most probably such researches will never be conducted due to ethical reasons. The included studies were restricted to those published in English which lead to exclusion of non-English studies with their evidence base. This may increase the likelihood of selection bias. Also, there was point of limitation and unavoidable weaknesses. All of the studies were in Sweden. And we could do nothing to overcome all these point. Wide range of the dates of publications of the included studies, almost five decades, increases the validity and significance of the results.

Analysis and investigation

A total of six studies with nine data points are comprised in this meta-analysis of LADA and sugary beverages consumption [20–25]. We used STATA (version 9.0; Stata Corp, College Station, TX, USA) to attain instantaneous relative risks employing random effects models as well as fixed effects models designed from the logarithm of the relative risks and matching 95% confidence intervals of the separate studies [20–27]. A random-effects model was used primarily because it integrates the constituents of variance within the study itself and also between the studies. Egger's test was acknowledged to be employed in case of heterogeneity between studies and it's also considered to be the more conventional method [28]. We assessed the heterogeneity significance of the results throughout our selected studies by the application of Cochrane Q test, in spite of the presence of lack of sensitivity. We followed Cochrane Q test by an I^2 statistical analysis which embodies the proportion of whole disparity across studies because of inter-study heterogeneity [29]. Sensitivity analysis has been conducted to avoid heterogeneity, which might occur as a result of the total calorie intake modification which includes a follow up procedure and other potential mediators. We used these combined mediators as conjecturers and forecasters of effect in the meta-regression analyses. Those mediators were likely be able to influence the association between SBC and LADA, so we are therefore obliged to adjust all of these mediators to weaken and lessen the effect. We used a visual assessment of the Begg funnel plot and applied the Begg and Egger analysis to evaluate and appraise any possibility for publication bias [30, 31]. Generally case-control studies can study rare diseases which have multiple risk factors for one disease as they are relatively cheap, quick and easy to design due to retrospective recall because of the already existing data. However, this design could not study several diseases, rare exposures or even estimate the incubation period between risk factor of the disease in question, and disease itself. Neither could it measure the risk directly nor even the occurrence rates including the incidence and prevalence of the same. Relative risk could not be calculated but the Odds ratio could be. Thus given everything mentioned above, in terms of strength of association, case control studies showed the same strength as cohort because both were analytical studies [32].

Results and findings

Characteristics of all the study population included within our meta-analyses are presented in Table 1. Each research study assessed the risk of SBC in development of LADA (nine data points) [20–25], comprising males and females of the Caucasian population from Sweden and all of whom were adults. Regarding all the selected case-control studies, each of which were compared via a retrospective recall of previous exposure to the risk factor, which was sugary beverages consumption, including coffee and alcohol. Cases were matched with controls in relation to number of participants and demographic characteristics and was conducted by interviews and structured questionnaires which included food frequency questionnaires (FFQs). There were 15027 participants involved with and 1862 patients with LADA. The research articles assessed the dietary intake [20–25] revealed effect estimations that were not adjusted for total calorie intake or measures of BMI. According to the data from those six articles, the shared odds ratio [OR] for LADA was 1.37 [95% CI 1.23–1.52]). Overall P value 0.001, $I^2 = 73.1\%$, for the

Table 1. Spreadsheet characteristics of studies entailed SBC and risk of LADA and type 2 diabetes mellitus (n = 6)

Ref.	Population	Age range (years)	Study design	Dietary assessment method	Beverage used in study	Potential mediators	Results	Conclusion
Löfvenborg et al. 2019 [20]	Sweden 386 LADA cases 1545 LADA controls 1253 type 2 diabetes mellitus cases	40–69	Observational analytical Case-control study	Diet history	High sweetened beverage intake	Age, sex, BMI, family history, smoking and education	OR (95% CI) between extreme quartiles of median SBC (0 *vs.* 143 g/day): 1.67 (0.98–2.87); P trend < 0.01	High sweetened beverage intake encompass autoimmune forms of diabetes
Rasouli et al. 2014 [21]	Sweden 250 LADA cases 1012 LADA controls 764 type 2 diabetes mellitus cases	45–64	Observational analytical Case-control study	FFQs	Coffee intake	High-risk HLA genotypes, age, sex, BMI, family history, smoking and education	Men: OR (95% CI) between extreme quartiles of SBC (> 1–8-oz serving/ /day *vs.* < 2–8-oz servings/day): 1.09 (0.89–1.33); P trend < 0.68 Women: OR (95% CI) between extreme quintiles of SBC: 1.17 (0.94–1.46); P trend < 0.05	Coffee intake is positively associated with LADA among carriers of high-risk HLA genotypes
Löfvenborg et al. 2014 [22]	Sweden 245 LADA cases 990 LADA controls 759 type 2 diabetes mellitus cases	38–65	Observational analytical Case-control study	133 item FFQs	Intakes of sweetened beverages	Genetic susceptibility conferred by genotypes of HLA, FTO or TCF7L2	OR (95% CI) between extreme quartiles of SBC (< 1 serving/month *vs.* < 1 serving/day: 1.83 (1.42–2.36); P trend < 0.001	High intakes of sweetened beverages increase the risk of both LADA and type 2 diabetes mellitus
Rasouli et al. 2012 [23]	Sweden 140 LADA cases 1841 type 2 diabetes mellitus cases	42–57	3 cross-sectional surveys	68 item FFQs	Alcohol consumption	Age, sex, race, education, center, total calories, smoking, physical activity, intake of meat, dairy, fruits and vegetables, whole grains, and refined grains	OR (95% CI) between extreme quartiles of SBC (0 *vs.* > 1 serving/day): 0.15 (0.12–1.42); P trend > 0.65	Alcohol consumption may improve insulin sensitivity and reduce the risk of type 2 diabetes LADA
Löfvenborg et al. 2016 [24]	Sweden 357 LADA cases 1136 LADA controls 1371 type 2 diabetes mellitus cases	39–63	Observational analytical Case-control study	FFQs	Coffee	BMI, physical activity, family history of diabetes, postmenopausal hormone use, alcohol use, smoking, and total energy intake	OR (95% CI) between extreme quintiles of SBC: (> 1–12-oz serving/ /month *vs.* 2–3 12-oz servings/day): 1.31 (0.99– –1.74); P trend < 0.001	Coffee may promote autoimmunity and possibly even increase the risk of autoimmune diabetes
Rasouli et al. 2018 [25]	Sweden 484 LADA cases 1609 LADA controls 885 type 2 diabetes mellitus cases	37–67	Observational analytical Case-control study	FFQs	Alcohol consumption	Physical activity, family history of diabetes, smoking, postmenopausal hormone use, oral contraceptive use, cereal fiber, magnesium, trans fat	OR (95% CI) between extreme quintiles of SBC (< 1 12-oz serving/ /month *vs.* > 2–12-oz servings/day: 1.24 (1.06–1.45); P trend < 0.002	Moderate alcohol consumption reduces risk of both type 2 diabetes and autoimmune diabetes

difference between extreme quantiles of SBC indicates an additional risk of 26% related with increased SBC.

Even though all researches apart from two [21, 23] exhibited positive associations and significant correlations between SBC and LADA, there was considerable heterogenetic difference among them in the analysis, where the P value was calculated for the test of heterogenetic difference for LADA: 12–66% (95% CI 31–83), however P value, was 0.003. Rasouli et al. [23] performed as a cross sectional study survey assessing LADA and expressed non-significant negative association [23]. If we exclude this research from our analysis, the heterogenetic difference will be reduced slightly, 12–62% (95% CI 17–82), however the P value, was 0.01. On the other hand, the remaining studies showed clear significant positive association, except for one study [21] which also provided clear non-significant negative association between alcohol and LADA among men. However, it did conclude that alcohol could be a potential protective factor against LADA in women, due to the significant negative correlation ($P < 0.68$ for men [21] $P < 0.05$ for women [21] $P < 0.65$ [23]). In spite of this condition, findings from the meta-regression analysis did not find that the noted variation in either study [21, 23] to make a significant difference.

The case control study by Löfvenborg et al. [22], which displays a marginal significant positive correlation, has the smallest number of contributors and significantly lower amounts of SBC intake comparative to the other studies(median SBC is 143 g/day in uppermost quartile of intake, where intake of one 12--oz serving equal to 336 g). Exclusion of this research from the analysis did not lessen heterogeneity, as was expected, given its low statistical significance and small proportion weight (P value, test for heterogenetic difference 0.002). However the research done by Rasouli et al. [25], which had the biggest significance and which used frequent measures of SBC, described the robust estimation. Exclusion of this research from the mutual analysis has decreased heterogenic difference to a marginal significance (P value, test for heterogenic difference 0.05; I^2 51% [95% CI 0–78%]).

Assessments for publication bias usually depend on the supposition that a few studies with big variances may be more susceptible to publication bias, in comparison to large research studies. A visual review of the Begg funnel plot (accompanying Fig. 2), where by the standard error of log the relative risk (putting in consideration the study size) from each research was strategized against the log relative risk (effect of treatment), exhibited balance about the plot, suggestive of an impossible bias of publication, even though values for LADA may not be mostly helpful and informative because of the small number of studies comprised within the analysis. Studies with a big standard of error and great effect may recommend the existence of what is called "a small-study effect". In other words, the propensity of small research studies in the meta-analysis to show the big treatment effects) (P value for LADA was 0.75 in the studies of both Begg and Egger [28, 30, 31], Fig. 2).

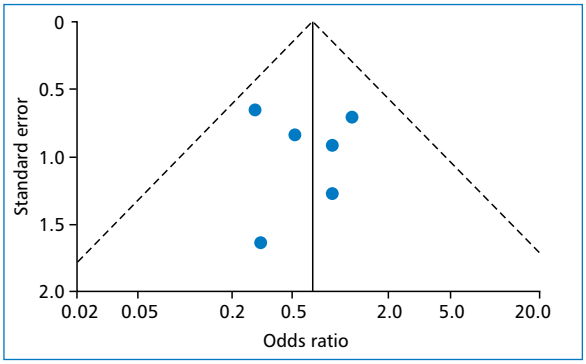

Figure 2. Funnel plot for assessment of publication bias

Findings from our analysis of sensitivity in which both calorie, and BMI, adjusted coefficients were omitted [20, 22] revealed a slight escalation in risk of LADA with a pooled relative risk of 1.28 and 95% CI (1.13–1.45). This is with regards to the random-effects-model and on the other hand, relative risk of 1.250 (1.18–1.34) regarding the fixed-effects-model. There was a larger increase which was distinguishable in the dose-response-meta-analysis and when we excluded those studies [20, 22]: relative risk was 1.350 (1.14–1.59) and this regarding the random-effects-model. On the other hand, the relative risk of 1.180 (1.12–1.24) with regards to the fixed-effects-model. However findings from the meta-regression did not adjust for calorie intake as it was not considered to be an important mediator of outcome ($P = 0.380$). Further analysis of sensitivity was not conceivable for studies of LADA because they are too scarce and yet, both studies that did adjust for those mediators of outcome had borderline insignificant associations [20, 22], whilst the research that had shown unadjusted estimations also showed a significant positive correlation [21].

Discussion and conclusion

As can be deduced from the presented meta-analysis there is a clear association between SBC and risk of LADA. This is based upon the coefficients from five case-control studies and one cross sectional survey, which involved 15027 contributors and 1862 patients with LADA. Contributors in the uppermost group of SBC

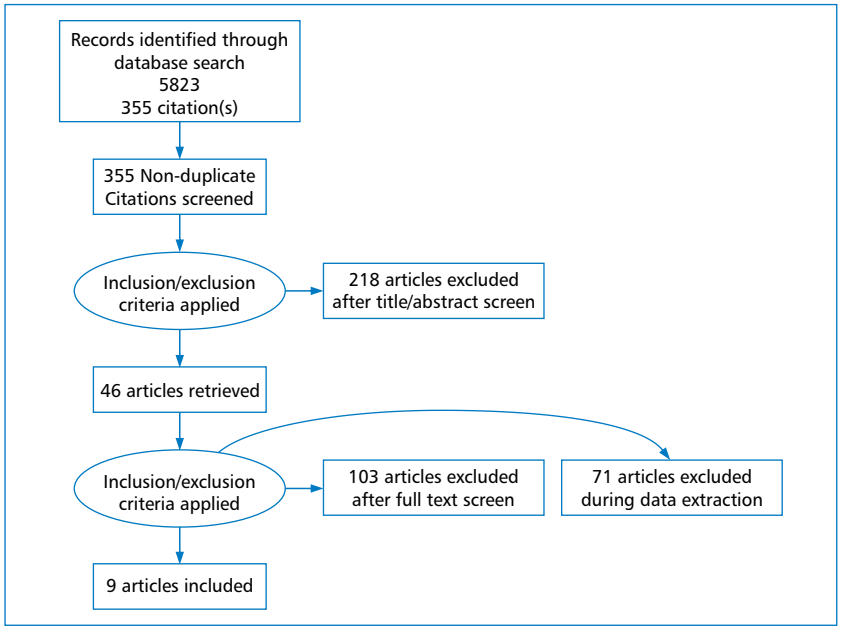

Figure 3. PRISMA diagram for selected studies

intake had a 20% more risk of developing LADA than those in the lowermost group of SBC intake.

Since we matched the extreme quantiles of SBC, mostly zero or one serving per month against one or two servings per day, groups of intake between the studies were not consistent or homogenous. Consequently, it is likely that a random biased classification fairly weakened the mutual estimation; though, findings were analogous to the dose-response-analysis, which used data from all groups. For those studies that did not outline a size of serving, an average serving of (12 Oz) was presumed, which may overestimate or undervalue the experiential SBC levels but ought not to substantially disturb our results. Certainly there is considerable difference in study designs and assessment of exposure, through the studies, which may elucidate the observable notch between heterogenic differences in studies we perceived. Meta-analysis is integrally not as strong as the distinct prospective cohort research but it is still beneficial in providing a holistic view about the effect size. Moreover, they also provide larger investigations and studies with less random disparities and more weight than the smaller studies. Publication bias is always seen as a prospective apprehension especially with meta-analyses. Nevertheless standard assessments and visual scrutiny of the funnel plots garnered no proof of any publication bias in our analysis.

All the research that was involved in our meta-analysis included well-thought-out adjustments for possible confusion by several factors such as diet and lifestyle, and mostly due to the persistence of positive association, signifying an autonomous effect of SBC. High levels of SBC could be indicator of a generally unhealthy diet as they lean towards the inclusion of other factors such as, ingestion of high saturated and trans-fatty acids and a low fiber intake [12]. So, an imperfect adjustment for several diet and lifestyle factors could possibly overstate the strength of the positive correlation between SBC and risk of LADA. However, the consistency of results from these different studies decreases the probability that an enduring variable is responsible for the results. Longitudinal studies assessing diet and the risk of chronic disease may similarly be exposed to inverse causality, i.e., persons alter their diet due to subclinical disease symptoms or associated obesity, which may result in false associations [26]. Though it is not imaginable to totally remove these factors, studies with long periods of follow up and frequent measurements of nutritional intake have a tendency to be less susceptible to this process.

In a few studies, LADA was evaluated by self-assessment; yet, it has been demonstrated in confirmation studies that self-reporting of LADA is highly precise according to the review of medical records [26]. The bulk of research studies have used validated Food Frequency Questionnaire to assess SBC, which is the strongest technique for assessing a personal average dietary consumption associated with other valuation methods such as the 24 hour dietary recall [27]. However, errors of measurement in dietary assessment are always unavoidable, but because the studies we deliberated are case-control in design, faulty classifica-

tion of SBC perhaps does not vary by case status. This non differential faulty classification of exposure may undervalue the real association between SBC and risk of such consequences.

SBC are thought to cause obesity due to their high supplementary sugar content, low compensatory water intake, reduced satiety and inadequate compensatory reduction in calorie intake during mealtimes which causes a positive energy balance and thus the body stores the extra food as fats [7, 8]. Even though, SBC increases the risk of LADA, partially due to their participation in weight gain, an autonomous effect may also come from the increased amounts of fast absorbable carbohydrates as extra sugars, used as flavors in beverages. The results by Löfvenborg et al. [20] suggested that nearly half of the consequences of SBC on LADA were arbitrated through obesity. In a recent longitudinal research which followed 88,000 females for 24 years, who were consuming 2 servings per day and had a 34% more risk of coronary insufficiency in comparison to occasional consumers after adjustment for other potential mediators (relative risk 1.35, and 95% CI of 1.1–1.7, where P-value < 0.01) [33]. Further adjustement of potential mediators like BMI and total calorie intake, weakened the associations, however they were still statistically significant, indicating that the effect of SBC is not fully mediated by those factors. SBC has been proven to increase blood sugar and insulin levels quickly and intensely [34] and if frequently used in big quantities, will undoubtedly lead to a high dietetic glycemic load. High glycemic load (GL) nutrition will lead to glucose intolerance and insulin resistance mostly among obese people [9] and can raise the levels of inflammatory mediators such as the C-reactive protein, which are associated with the risk of autoimmune beta-cell dysfunction [35]. Results from other studies showed that a high nutritional GL can also magnify the risk of acquiring cholesterol gallstones which are associated with autoimmune insulin resistance [36].

Endogenic compounds in sugary beverages, such as progressive glycation-end-products, created during the procedure of adding caramel to soda beverages like cola might also influence the patho-physiological mechanisms associated with diabetes [37]. Modifications in taste preferences and quality of diet, induced by SBC, might also circuitously participate in increasing the risk of development of diabetes [5]. Interim experimental researches recommend that fructose, which is an essential component of fructose/sucrose corn syrup in fairly equivalent amounts, may lead to predominantly metabolic adverse effects when compared with glucose. This is because fructose is otherwise metabolized to lipids inside the liver, causing increased biochemical processes of creating fatty acids from acetyl CoA that are formed from a number of different mechanisms within the hepatic cell, leading to high levels of triglycerides, decreased high density lipoproteins, development of dyslipidemia, and also insulin resistance [38]. In brief, this meta-analysis has not shown that excessive SBC is associated with the risk of development of LADA. It offers moderate evidence to support the restricted intake of these drinks and the use of healthy substitutes instead like water to which will decrease the risk of chronic diseases. Nevertheless, there were no long term randomized studies, with sufficient follow-up period, to show potential relationship between sugary beverages and LADA, and probably such studies will never be conducted due to ethical reasons. So, no definite conclusions could be drawn due to heterogeneous data from low quality researches and the analysis was based on observational and case-control studies only. So the authors were urged to elaborate on the fact that no definite conclusions could be drawn.

Summary

Only six research papers worldwide addressed the relation between consumption of sugary beverages and the development of latent autoimmune diabetes in adults. These articles had contradictory findings regarding this risk factor. We conducted a systematic review and meta-analysis to establish statistical significance across studies that might otherwise seem to have conflicting results. This will increase the validity and reliability of information and any observed differences. This is the first systematic review and meta-analysis comparing this correlation, to find a clear significant association between sugary beverages consumption and latent autoimmune diabetes in adults.

Ethical approval and consent to participate

The authors certify that the guidelines of the PRISMA 2009 statement have been adopted.

Competing interests and conflict-of-interest statement

The authors certify that they have no conflicts of interest to declare including but not limited to financial, consultancy, advisory, institutional, and other relationships that might lead to a possible bias or misconstrue the results and/or conclusions of this research.

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The effect of low dose glargine U 300 on uncontrolled type 2 diabetes mellitus. An observational study in Indian patients

ABSTRACT

Introduction. Insulin therapy plays an important role in the management of diabetes mellitus. The primary goal of insulin therapy is to achieve the best possible glycemic control without hypoglycemia. First-generation basal insulin (BI) analogues, such as insulin glargine 100 U/ml (Gla-100) and insulin detemir (IDet), provide more prolonged and stable activity than neutral protamine Hagedorn (NPH) insulin, with a lower risk of hypoglycemia. Insulin glargine 300 U/mL (glargine U 300) is a long acting basal insulin analogue approved for the treatment of diabetes mellitus. Insulin glargine 300 U/mL has a more stable and prolonged pharmacokinetic/ /pharmacodynamics profile than insulin glargine 100 U/mL, with a duration of glucose-lowering activity exceeding 24 h. Although the average daily insulin dose was higher, hypoglycemia episodes were lower in patients treated with Gla-300 compared with those treated with Gla-100. This is due to a more extended time action profile than Gla-100 resulting in a more stable and sustained glycemic control. The formulation of Gla-100 delivers the same amount of Insulin as Gla-300, in a third of the injection volume. It is essential to determine whether the clinical benefits of hypoglycemia reduction observed with insulin glargine in RCTs translate into a real-life clinical practice setting.

Materials and methods. Fifty patients diagnosed with type 2 diabetes mellitus with uncontrolled plasma glucose levels (HbA_{1c} of > 7.5) who were on oral hypoglycemic agents, premix insulin and basal bolus therapy were enrolled into the study and started on glargine U 300 at a dose of 0.2 IU/kg and analysed for glycemic and kidney function parameters. The patients were followed up at 3 and 6 months post treatment.

Results. All glycemic control parameters decreased significantly with almost a 50% decline in both FBS and PBS from baseline to 6 months. The HbA_{1c} decreased significantly from baseline to 3 months and 6 months post treatment by 18% and 29% respectively. All biochemical parameters were found to be statistically significant in both groups.

Conclusions. In patients with uncontrolled type 2 diabetes, switching from either OHAs or insulin to Gla-300 improves glycemic control, with a low incidence of hypoglycemia. These results confirm the effectiveness and safety of Gla-300 in a real-world setting and show that Gla-300 is a suitable therapy option for patients with diabetes. (Clin Diabetol 2020; 9; 2: 128–133)

Key words: type 2 diabetes mellitus, insulin analogues, glargine U 300, hypoglycemia, kidney function parameters, HbA_{1c}, fasting blood sugar, post prandial blood sugar

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Introduction

Diabetes mellitus is a metabolic disease marked by elevated plasma glucose. In type 2 diabetes mellitus (T2DM), the pancreas often continues to produce insulin, sometimes even at higher-than-normal levels,

especially early in the course of the disease. However, the body develops resistance to the effects of insulin, so there is not enough insulin to meet the body's needs. As type 2 diabetes progresses, the insulin-producing ability of the pancreas decreases. T2DM tends to develop in adults, and it results from insulin resistance accompanied by a gradual decline in β-cell function. In T1 and T2DM, impaired glucose regulation leads to chronic hyperglycemia, which induces a variety of vascular complications.

The global diabetes epidemic has reached alarming proportions, particularly in Asia. In 2017, prevalence among adults ranged from 7.7% to 13.7% in this region with more than 1 million diabetes-related deaths [1].

Although there is no cure for diabetes yet, it can be treated and effectively controlled. The goals of diabetes management are to keep the plasma glucose levels as near to optimal levels as possible by balancing diet with medication and activity. Other co-morbidities such as hypercholesteremia, hypertension and dyslipidemia should also be controlled. Despite the introduction of numerous anti-hyperglycemic medications, many patients with T2DM require insulin, and basal insulins continue to be frequently used either as first-line insulin treatment or as part of multiple daily injection regimens. Current American Diabetes Association (ADA) guidelines recommend a HbA_{1c} target of 7.0% [2].

Insulin therapy plays an important role in the management of diabetes mellitus. The primary goal of insulin therapy is to achieve the best possible glycemic control without hypoglycemia. First-generation basal insulin (BI) analogues, such as insulin glargine 100 U/ml (Gla-100) and insulin detemir (IDet), provide more prolonged and stable activity than neutral protamine Hagedorn (NPH) insulin, with a lower risk of hypoglycemia [3]. It is essential to determine whether the clinical benefits of hypoglycemia reduction observed with insulin glargine in RCTs translate into a real-life clinical practice setting.

Insulin glargine 300 U/mL (glargine U 300) is a long acting basal insulin analogue approved for the treatment of diabetes mellitus. Insulin glargine 300 U/mL has a more stable and prolonged pharmacokinetic/pharmacodynamics profile than insulin glargine 100 U/mL, with a duration of glucose-lowering activity exceeding 24 h. Clinical trial data has shown that, following injection, Gla-300 has slower rate of release into the surrounding tissues when compared with Gla-100. This results in a more even plasma concentration of the drug and also better glucose lowering effect resulting in a longer duration of action that fully covers a 24-h dose period with a single injection [4].

The efficacy and safety of Gla-300 was studied extensively in the EDITION series of clinical trials, which compared Gla-300 with Gla-100 in patients with T1D or T2D, with differing treatment backgrounds. These clinical trials demonstrated that hemoglobin A_{1c} level decreased by equivalent amounts with Gla-300 and Gla-100 treatment, regardless of the type of diabetes or whether patients were insulin naive or being switched from oral treatment with another basal insulin.

The most common and serious adverse effect of insulin treatment, hypoglycemia remains a key unmet medical need in insulin-treated diabetes. Insulin related hypoglycemia and insulin-use errors account for a significant number of hospital visits. The risk of hypoglycemic episodes increase in the elderly and patients with renal impairment. Although the average daily insulin dose was higher, hypoglycemia episodes were lower in patients treated with Gla-300 compared with those treated with Gla-100 [5, 6]. This is due to a more extended time action profile than Gla-100 resulting in a more stable and sustained glycemic control. The formulation of Gla-100 delivers the same amount of Insulin as Gla-300, in a third of the injection volume.

The current study was envisaged to evaluate the effects of Gla-300 when prescribed in uncontrolled diabetes including to assess effects on markers of kidney function along with parameters determining glycemic control over a period of six months prompting the need for a more careful management of insulin therapy.

Materials and methods

This was an observational study. 50 patients diagnosed with type 2 diabetes mellitus with uncontrolled plasma glucose levels (HbA_{1c} of > 7.5) who were on oral hypoglycemic agents premix insulin or basal bolus regime, were enrolled into the study. Initially all subjects were started on glargine U 300 at a dose of 0.2 IU/kg. Patients treated in diabetes clinic of Ruby General Hospital and principal investigator had complete oversight of the patients during the six month study period. The dose was titrated up to 0.4 IU/kg over a period of two weeks according to self-monitoring for blood glucose (SMBG) diary. Patients who were on premix insulin were converted to basal bolus regime with the basal insulin being glargine U 300 and bolus insulin with human insulin Actrapid. In patients who were already on basal bolus insulin their basal insulin was changed to glargine U300, keeping bolus dose unaltered. Patients were instructed to do SMBG per day and maintain a diary. The 5 SMBGs recommended were 3 premeals, 1 bedtime and 1 at 3 am and SOS measurement if the subjects experienced an episode of hypoglycaemia. Hypoglycemia was defined as glucose levels below 70 mg/dL. Patients were instructed to look out for symptoms suggestive of hypoglycemia

Table 1. Demographics of the study population

	All subjects (N = 48)	Male (N = 25)	Female (N = 23)
Age in years (mean)	58.42 ± 9.65	60.4 ± 10.79	56.26 ± 7.92
Height [cm]	162.94 ± 9.22	166.84 ± 8.35	158.7 ± 8.34
Weight [kg]	66.35 ± 11.67	69.76 ± 11.65	62.65 ± 10.74
BMI [kg/m^2]	25.03 ± 3.99	25.19 ± 4.3	24.84 ± 3.73

BMI — body mass index

Table 2. Comparison of changes in biochemical parameters in 48 subjects at baseline, 3 months and 6 months follow up

Age (N = 48)	BMI	Parameters	Baseline	3 months	6 months	Change in 3 months (%)	Change in 6 months (%)	P-value
58.42 ± 9.65	25.02 ± 4	ACR	46.5 ± 44.1	33.54 ± 27.82	24.31 ± 17.79	–27.87	–47.72	0.00003
		eGFR	78.06 ± 13.54	81.33 ± 12.44	83.58 ± 12.36	4.19	7.07	< 0.00001
		FBS	213.73 ± 71.92	138.75 ± 29.66	112.04 ± 25.25	–35.08	–47.58	< 0.00001
		PPBS	345.46 ± 91.63	229.38 ± 49.25	178.73 ± 21.69	–33.60	–48.26	< 0.00001
		HbA_{1c}	10.03 ± 0.91	8.24 ± 0.67	7.1 ± 0.24	–17.88	–29.28	< 0.00001

BMI — body mass index; ACR — albumin creatinine ratio in spot urine sample; eGFR — estimated glomerular filtration rate by CKD-EPI formula; FBS — fasting blood sugar; PPBS — 2 hour post prandial blood sugar

such as dizziness, excessive sweating, reeling of head, confusion. The target glucose levels were between 140–180 mg/dL. Glargine U 300 was titrated to a target pre-prandial glucose concentration of 100–130 mg/dL and postprandial glucose concentration of 140––180 mg/dL based on CBG levels assessed on a weekly basis for two weeks at the clinic. Patients with type 1 diabetes, pregnant or lactating mothers, allergic to insulin or having estimated glomerular filtration rate (eGFR) of ≤ 30 were excluded. Demographic details, details of diabetes such as duration, treatment and diabetes education received etc. were collected from the patients. Two subjects were lost to follow up during the course of the study. One subject died due to bronchogenic carcinoma which was not related to insulin. The other subject dropped out because she could not afford the expenses involved in the therapy. The primary objective of the study was to evaluate the change in the parameters of fasting blood sugar, post prandial blood sugar and HBA_{1c} from baseline to 3 months and 6 months post treatment. The secondary objective was to evaluate the change in the parameters of biochemical markers of renal function from baseline to 3 months and 6 months post treatment. The safety objective was to evaluate the episodes of hypoglycaemia during the study period.

This study was carried out on FDA approved insulin analogue among Indian patients with diabetes. This study was conducted in accordance to the Declaration of Helsinki (1975 as revised in 2013).

Statistical analysis

All data analysis was carried out as per a comprehensive pre-planned statistical analysis plan. The intention-to treat analysis of the primary study end point was performed on all randomly allocated participants who satisfactorily complete all follow up visits. To evaluate the overall effects of glargine U 300 on markers of glycemic control and chronic kidney disease, the mean changes from baseline to 3 months and 6 months post treatment is calculated. P-value 0.05 was defined as the level of statistical significance. Data is expressed as mean ± SD for continuous variables and as percentage for categorical variables.

Results

Table 1 summarizes the demographic characteristics of the patients. The average age of the subjects in the study was 58.42 ± 9.65 years. The average age of males were higher compared to the females (60.4 ± 10.79 *vs.* 56.26 ± 7.92 years). The average height of subjects in the study was 162.94 ± 9.22 cm with males comparatively taller than females (166.84 ± 8.35 *vs*. 158.7 ± ± 8.34 cm). The average weight of subjects in the study was 66.35 ± 11.67 kg with males comparatively heavier than females (69.76 ± 11.65 *vs*. 62.65 ± 10.74 kg). The average BMI of the subjects was 25.03 ± 3.99.

Table 2 summarizes the biochemical parameters (ACR, eGFR, FBS, PBS and HbA_{1c}) for all enrolled patients compared from baseline to the third month and the sixth month using repeated measures ANOVA and

Table 3. Baseline characteristics by categories of ACR (< 30 mg/g i.e., normal) irrespective of BMI status and % change after treatment received

Age	BMI	Parameters N = 23	Baseline	3 months	6 months	Change in 3 months (%)	Change in 6 months (%)	P-value
59.48 ± 10.14	24.35 ± 3.24	ACR	17.48 ± 5.98	25.57 ± 27.51	13.13 ± 4.59	46.28	–24.89	0.0355
		eGFR	75.26 ± 11.1	79.74 ± 9.88	82.35 ± 10.46	5.95	9.42	< 0.00001
		FBS	204.43 ± 49.71	138.65 ± 34.10	113.96 ± 33.66	–32.18	–44.25	< 0.00001
		PPBS	328.91 ± 85.93	227.09 ± 49.2	177.26 ± 27.32	–30.96	–46.11	< 0.00001
		HbA_{1c}	9.88 ± 0.64	8.02 ± 0.52	7.05 ± 0.18	–18.83	–28.64	< 0.00001

BMI — body mass index; ACR — albumin creatinine ratio in spot urine sample; eGFR — estimated glomerular filtration rate by CKD-EPI formula; FBS — fasting blood sugar; PPBS — 2 hour post prandial blood sugar

Table 4. Baseline characteristics by categories of ACR (30–300 mg/g i.e., microalbuminuria) irrespective of BMI status and % change after treatment received

Age	BMI	Parameters N = 25	Baseline	3 months	6 months	Change in 3 months (%)	Change in 6 months (%)	P-value
57.44 ± 9.28	25.64 ± 4.57	ACR	73.2 ± 47.19	40.88 ± 26.55	34.6 ± 19.25	–44.15	–52.73	< 0.00001
		eGFR	80.64 ± 15.23	82.8 ± 14.46	84.72 ± 14	2.68	5.06	0.0006
		FBS	222.28 ± 87.77	138.84 ± 25.63	110.28 ± 14.26	–37.54	–50.39	< 0.00001
		PPBS	360.68 ± 95.76	231.48 ± 50.21	180.08 ± 15.28	–35.82	–50.07	< 0.00001
		HbA_{1c}	10.18 ± 1.1	8.44 ± 0.73	7.14 ± 0.28	–17.09	–29.86	< 0.00001

BMI — body mass index; ACR — albumin creatinine ratio in spot urine sample; eGFR — estimated glomerular filtration rate by CKD-EPI formula; FBS — fasting blood sugar; PPBS — 2 hour post prandial blood sugar

were found to be statistically significant. There was a reduction of 27% and 47% in albumin creatinine ratio from baseline to 3 months and 6 months respectively. Similarly the eGFR increased by 4% and 7% from baseline to 3 months and 6 months respectively. All glycemic control parameters decreased significantly with a 47% and 48% decline in FBS and PPBS from baseline to 6 months respectively. The HbA_{1c} decreased significantly from baseline to 3 months and 6 months post treatment by 17% and 29% respectively.

Subjects were categorized into mildly increased and moderately increased groups based on the ACR values, assuming a cut of level of (< 30 mg/g) irrespective of their BMI status with 23 and 25 subjects respectively. All biochemical parameters were found to be statistically significant in both groups. Although there was an increase of 46% in albumin creatinine ratio from baseline to 3 months the values decreased at 6 months. Similarly the eGFR increased by 5% and 9% from baseline to 3 months and 6 months respectively. All glycemic control parameters decreased significantly with a 44% and 46% decline in both FBS and PBS from baseline to 6 months. The HbA_{1c} decreased significantly from baseline to 3 months and 6 months post treatment by 18% and 28% respectively (Table 3).

Biochemical parameters for ACR levels of moderately increased groups irrespective of BMI status were evaluated. There were statistically significant differences in all parameters from baseline to 3 months and 6 months (Table 4).

Biochemical parameters for overweight subjects according to the BMI were analyzed for ACR levels of moderately increased groups. Although there were statistically significant differences in all parameters from baseline to 3 months and 6 months, the eGFR in the moderately increased group was found to be statistically not significant (Table 5).

Comparison of parameters in subjects receiving Insulin along with oral hypoglycemic agents (OHAs) is presented above. The biochemical parameter of ACR and eGFR along with glycemic parameters HbA_{1c} and FBS and PBS reduced from baseline to study end and this reduction was statistically significant (Table 6).

The number of subjects at various levels of intensity of risk for CKD were analyzed considering both eGFR and UACR values. It was found that there was a decline in the number of subjects in the moderately increased risk by the end of the study, whereas the number of subject in the low risk category increased from 21 to 38 towards the end of 6 months of the study (Table 7).

Table 5. Baseline characteristics by categories of ACR (30–300 mg/g i.e., microalbuminuria) and BMI status of overweight and % change after treatment received

Age	BMI	Parameters N = 13	Baseline	3 months	6 months	Change in 3 months (%)	Change in 6 months (%)	P-value
57.54 ± 9.97	27.92 ± 1.26	ACR	89.85 ± 57.36	50.62 ± 33.15	41.77 ± 20.7	–43.66	–53.51	0.0003
		eGFR	84.92 ± 13.33	86.77 ± 12.13	88.77 ± 12.45	2.18	4.53	0.1128
		FBS	192.23 ± 43.32	130 ± 19.85	105.85 ± 9.81	–32.37	–44.94	< 0.00001
		PPBS	347.69 ± 52.57	222.08 ± 22.86	181.08 ± 11.82	–36.13	–47.92	< 0.00001
		HbA_{1c}	10.08 ± 0.46	8.43 ± 0.33	7.15 ± 0.2	–16.37	–29.07	< 0.00001

BMI — body mass index; ACR — albumin creatinine ratio in spot urine sample; eGFR — estimated glomerular filtration rate by CKD-EPI formula; FBS — fasting blood sugar; PPBS — 2 hour post prandial blood sugar

Table 6. Comparison of biochemical parameters in insulin (once daily glargine U 300) + oral hypoglycemic agents (OHAs) group at baseline, 3 months and 6 months follow up

Age	BMI	Parameters N = 43	Baseline	3 months	6 months	Change in 3 months (%)	Change in 6 months (%)	P-value
58.42 ± 9.65	25.02 ± 4	ACR	46.5 ± 44.1	33.54 ± 27.82	24.31 ± 17.79	–27.87	–47.72	0.00003
		eGFR	78.06 ± 13.54	81.33 ± 12.44	83.58 ± 12.36	4.19	7.07	< 0.00001
		FBS	213.73 ± 71.92	138.75 ± 29.66	112.04 ± 25.25	–35.08	–47.58	< 0.00001
		PPBS	345.46 ± 91.63	229.38 ± 49.25	178.73 ± 21.69	–33.60	–48.26	< 0.00001
		HbA_{1c}	10.03 ± 0.91	8.24 ± 0.67	7.1 ± 0.24	–17.88	–29.28	< 0.00001

BMI — body mass index; ACR — albumin creatinine ratio in spot urine sample; eGFR — estimated glomerular filtration rate by CKD-EPI formula; FBS — fasting blood sugar; PPBS — 2 hour post prandial blood sugar

Table 7. Comparison of no of subjects in term of risk at baseline, 3 months and 6 months follow up [7]

Intensity of risk	Baseline	3 Months	6 months
Low risk	21 (43.75%)	28 (58.33%)	38 (79.17%)
Moderately increased risk	27 (56.25%)	20 (41.67%)	10 (20.83%)
High risk	0	0	0
Very high risk	0	0	0

There were 5 (10%) patients who reported episodes of hypoglycaemia at various time points in the course of the study. None of the patients required hospitalization and were managed with oral glucose solution. The dose of their OHA or insulin was reduced.

Discussion

This observational study evaluated the efficacy and safety of glargine U 300 in patients with uncontrolled T2DM on oral hypoglycemic agents and/or insulin therapy. The subjects were switched to Gla-U 300 as they failed to respond to current line of management due to less than optimal glycemic control. The study aims to explore the effectiveness and safety of Gla-U 300 in eastern Indian population. All patients were initially started on a low dose of 0.2 units as against the western standard of 0.4 units, the rationale behind this approach is the lower BMI observed in Indian population. After adequate titration the patients were followed up at 3 months and 6 months. Glycemic control was significantly improved with a very low incidence rate of hypoglycemia and no significant weight change.

The switch over from basal/bolus insulin or oral hypoglycemic agents to Gla-300 has been studied in previous trials. The EDITION-2, a phase 3a randomized controlled trial and DELIVER-2 was a retrospective cohort study that used propensity score matching. After 3 and 6 months of therapy with Gla-300, there was significant reductions in mean HbA_{1c} by 17% and 28% respectively in male patients and by 18% and 29% respectively in male patients.

Biochemical parameters (ACR, eGFR, FBS, PBS and HbA_{1c}) from baseline to 3 months and 6 months post treatment were assessed using repeated measures of ANOVA and were found to be statistically significant. There was a reduction of 27% and 47% in albumin creatinine ratio from baseline to 3 months and 6 months respectively. Similarly the eGFR increased by 4% and 7% from baseline to 3 months and 6 months respectively. All glycemic control parameters decreased significantly with a 47% and 48% decline in FBS and PPBS from baseline to 6 months respectively. The HbA_{1c} decreased significantly from baseline to 3 months and 6 months post treatment by 17% and 29% respectively.

The majority of the patients i.e. 25 (50%) achieved their individual HbA_{1c} target of 7% by switching their basal insulin to Gla-300, while 24 (48%) achieved HbA_{1c} level of below 7.5%.

Adequate dose titration is essential after the initiation of an insulin regimen after careful evaluation of the patient's glycemic control, but maybe delayed in clinical practice resulting in prolongation of the duration of diabetes and other comorbidities. Most important barrier for dose titration are concerns about hypoglycemia. First-generation basal insulin analogues such as Gla-100 were shown to provide significant advantages over NPH insulin regarding the risk for hypoglycemic events and administration, and recently approved second-generation basal insulins such as Gla-300 afford even greater improvements. This was illustrated in our study wherein a good number of patients were still maintained on the low dose of 0.2–0.3 IU/kg (89.5%) and only 5 subjects required a dose titration to 0.4 units (10%). The number of hypoglycaemia episodes were also not significant with only 5 subjects (10.4%) reporting events none of which required hospitalization.

A major limitation of the current study was the small number of patients (n = 50) that were followed up until 6 months. The lack of a direct comparator is another major limitation, as no information on the outcomes with alternative lines of treatment could be compared. However, the study demonstrated consistent and sustained glucose control with Gla-300 and a significantly lower risk of hypoglycemia.

Conclusion

In patients with uncontrolled type 2 diabetes, switching from either OHAs or insulin to Gla-300 improves glycemic control, with a low incidence of hypoglycemia. These results confirm the effectiveness and safety of Gla-300 in a real-world setting and show that Gla-300 is a suitable basal insulin therapy option for patients with type 2 diabetes mellitus.

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Lower limb bilateral pyomyositis in a diabetic patient: case report and literature review

ABSTRACT

Pyomiositis is a bacterial infection of skeletal muscle tissue occurring mostly in immunocompromised patients, including diabetics. The main agents are Gram-positive cocci. We report a case of a 42 years old, male, diabetic, who presented the emergency room referring pain in thighs and inability to flex his legs. We performed a magnetic resonance which revealed extensive purulent collections in both lower limbs, thus confirming clinical suspicion, and allowing proper antibiotic treatment. In this case, we showed that imaging tests facilitate early diagnosis and treatment through direct location of the lesions, and guides invasive procedures such as biopsy and abscess aspiration when needed. (Clin Diabetol 2020; 9; 2: 134–137)

Key words: pyomyositis, diabetes mellitus type 2, magnetic resonance imaging

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Introduction

Scriba, in 1885, reported the first case of pyomyositis [1], as a primary acute bacterial infection of skeletal muscle's tissue [1, 2]; it occurs mainly in immunocompromised patients [3], malnurished [4] or, exceptionally, in immunocompetent patients in tropical areas [3]. In fact, it was originally described as a tropical disease and is now being seen, with increasing frequency, in temperate regions; pyomyositis holds a mortality rate of about 10% and may also be responsible for complications such as abscesses, septicemia, and shock [2].

The pathogenesis of primary pyomyositis remains unclear, although trauma and immune impairment can be predisposing factors [2]. It affects individuals, preferably during the first and second decades of life, and is more prevalent in men [1]; patients over 30 years of age usually have associated comorbidities [5], and the most described in the literature are human immunodeficiency virus infection [6], diabetes mellitus, malignant tumors, liver cirrhosis, renal failure, transplantation and use of immunosuppressive drugs, and intravenous drug abuse [1, 3]. Its initial signs and symptoms are usually subtle and can be easily overlooked or misdiagnosed as a more benign condition [2], specially erysipelas.

Pyomyositis can affect any muscle group, infecting by contiguity or hematogenous spread [1, 5]. The main agents are Gram-positive cocci [3], and the most common etiologic agent is *Staphylococcus aureus*, in up to 85–95% of the cases [1, 2, 5] — with increasing frequency of resistance to methicillin (MRSA) [2].

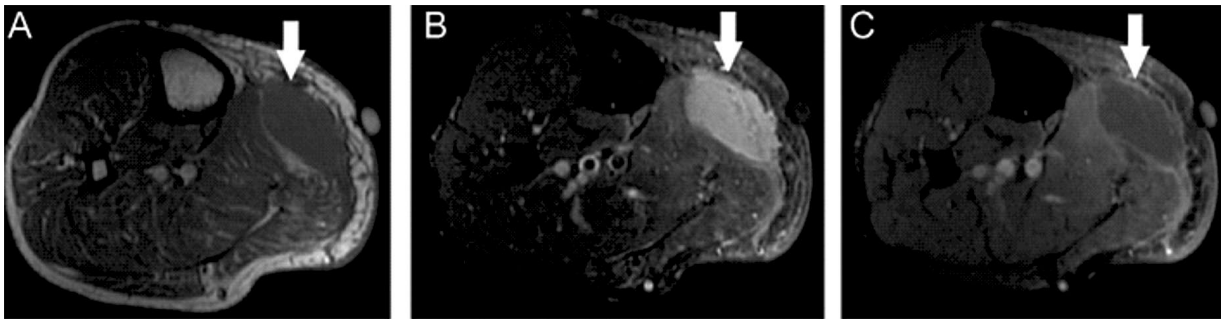

Figure 1. MRI of the right leg in the axial section T1-weighted sequence in **A**, T2 STIR in **B** and T1 sequence with contrast in **C** demonstrating a collection between the musculature and subcutaneous tissue with peripheral contrast enhancement (white arrows)

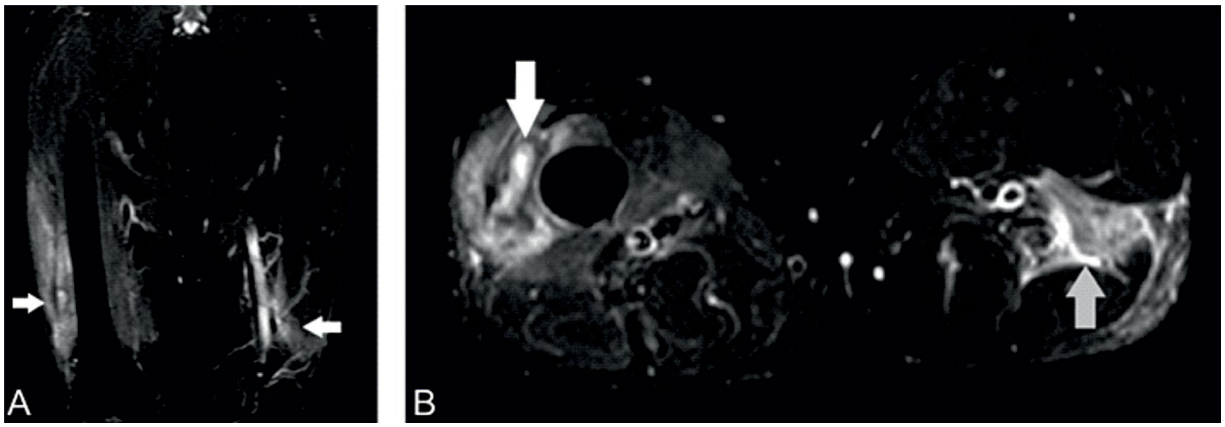

Figure 2. MRI of the thighs in the coronal section T2 STIR in **A** and axial section in **B**, demonstrating swelling of the right vastus lateralis and left femoral biceps muscles (white arrows in A) with collection in the right vastus medius muscle (white arrow in B), edema of the other muscles bellies with subfascial and peripheral fluid (gray arrow in B)

Case report

The patient is a 42 years old, Brazilian male, security agent, referred to the emergency department complaining of a acute pain of severe intensity on both thighs, restraining flexion of both lower limbs for the last seven days. He reported that a less severe pain had been present for over two months, and, one month earlier, he was hospitalized for 15 days due to erysipelas in his right leg — received analgesics without improvement at that time. He denied smoking or trauma, reporting, just social drinking; as a family background his father was diabetic and his mother had arterial hypertension. Physical examination of the patient showed a hyperemic, swollen and painful right leg, especially in the calf; his body temperature was 38.2° C pulse of 98 ppm and blood pressure of 150 over 80 mm Hg. Laboratory workup showed increased leukocyte count (18,452 hg/mm^3) and elevated c-reactive protein 15 mg/dL. Throughout this hospitalization, he was also diagnosed with type 2 diabetes mellitus and systemic arterial hypertension, having started treatment since then, and had good glucose control throughout hospitalization with metformin.

During the first hospitalization, a magnetic resonance image (MRI) of the right leg was performed (Figure 1 — description on the legend), but it might had been neglected. Given the progression of symptoms and severity of the clinical setting, a new MRI of both thighs was performed (Figure 2 — description on the legend), which showed purulent collection affecting both legs, rising the diagnosis of bilateral pyomyositis stage 2. Patient started to receive ceftriaxone plus teicoplanin empirically, and after 2 weeks was discharged with complete resolution.

Discussion

The diagnosis of pyomyositis can be challenging due to the nonspecific clinical signs at onset [2]. It may be confused with bone or joint infection processes, thrombophlebitis, panniculitis or systemic diseases [2]. The differential diagnosis includes muscle trauma, deep vein thrombosis, osteomyelitis, cellulitis, septic arthritis, and malignant tumors [2].

In the case of diabetic patients, it appears that muscular and circulatory disorders which, together with granulocyte dysfunction and decreased cellular immunity, increase the risk of developing pyomyositis [4]. Laboratory findings are nonspecific and the blood count may show leukopenia or leukocytosis with left deviation, depending on the etiologic agent and the patient immune response [1, 4]. There may be increased inflammatory markers and generally does not affect kidney function [3, 5]. Blood cultures are positive in less than 40% of patients and secretion cultures are positive in only 21–41% of cases [6].

Usually, it only affects one muscle group, but in 11% to 43% of patients may have widespread outbreaks in various muscles, being quadriceps muscle the most affected, followed by the gluteal and iliopsoas muscles [7]; iliopsoas pyomyositis occurs more commonly secondary to gastrointestinal or urinary tract infections [7].

Pyomyositis presents three evolutionary stages: invasive, purulent and late. The invasive stage is characterized by insidious onset, where only about 2% of affected patients seek medical support. The purulent stage occurs about 10–21 days after symptom onset and is characterized by the abscess location and, at this stage, about 90% of patients seek medical care due to the severity of the symptoms. If the disease is not identified and treated properly with antibiotics and/or surgical drainage, evolution occurs for the so-called late period associated with significant systemic involvement like septic embolization, septic shock, and even death [8]. Our patient had a bilateral phase 2 pyomiositis, due to an initial misdiagnosis of erysipelas, when he was still on initial phase.

Imaging tests such as ultrasound, computed tomography (CT) and magnetic resonance imaging (MRI) are useful to identify the number, size, extension, location of abscesses, and guide needle biopsy [9].

Ultrasound has high sensitivity for fluid collections detection; it may demonstrate initial muscle edema as a hypoechoic lesion with imprecise limits affecting one or more muscles that, with latter disease evolution, can lead to the formation of abscess [3].

CT scan shows enlargement and decreased attenuation of the affected muscle, with a blurring of surrounding fat. The involvement of a muscle group that is disproportionate to the involvement of the subcutaneous tissue helps distinguish myositis from primary cellulitis. Intramuscular collections can be observed and contrast material is used to help differentiate viable and necrotic muscles by demonstrating enhancement around the abscess [5, 7]. CT scan, as well as ultrasound, can help guide needle aspiration [3] and are also useful for surgical planning, with abscess drainage muscle followed by culture-guided antibiotics [5, 7].

MRI is the gold standard for the diagnosis by detecting early findings of muscles diffuse inflammation [5], and may also guide biopsy of the affected muscles [10]. MRI can show extension of infection and evaluates adjacent structures, such as joints, bone, and other surrounding soft tissues, differentiating pyomyositis from other differential diagnosis.

Treatment with antibiotics in conjunction with surgical drainage or suction, when applicable, is generally sufficient; prognosis is good with early diagnosis and appropriate treatment, depending on the underlying comorbities [11, 12]. Usually, one antibiotic with *S. aureus* coverage such as oxacillin, ciprofloxacin or cephalosporins is enough, but when dealing with immunocompromised patients, association of a amynoglicoside or even glycopeptides may be useful [5].

Untreated pyomyositis complications may include compartment syndrome, progression infection to septic arthritis, osteomyelitis and even death, which ranges between in up to 10% of the cases [5, 7]. In the long term, patients may experience weakness, and muscle dysfunction [5, 7].

In conclusion, pyomyositis may have subtle symptoms that are easily misinterpreted as signs of a more common and benign infection. Imaging tests facilitate early diagnosis and treatment through the direct location of the lesions and, when it's necessary, assist invasive procedures such as biopsy and abscess aspiration. The early recognition of the disease allows an earlier treatment preventing the development of complications.

Conflict of interest

We state that the authors have no conflict of interest.

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Long term management challenges in diabetic patients with rhino-orbito--cerebral mucormycosis

ABSTRACT

There has been a recent upsurge in prevalence of diabetes mellitus in developing world. This has resulted in exponential increase in the incidence of both communicable and non-communicable diseases. Some of the infections increase morbidity and mortality associated with diabetes. Rhino-orbital-cerebral mucormycosis (ROCM) is one of the fatal opportunistic infections in diabetes. There is little data published regarding the short and long term management of patients suffering from this invasive fungal infection. Hereby, we report few cases of ROCM with varied presentations and their short and long term follow up. These patients were initially treated with injectable amphotericin B and later followed up with oral posaconazole. (Clin Diabetol 2020; 9; 2: 138–140)

Key words: diabetes, posaconazole, rhino-orbital-cerebral mucormycosis

Introduction

As incidence of diabetes is rising globally, infections and sepsis are also imposing enormous burden on the disease outcome [1]. Mucormycosis is relatively uncommon and frequently fatal angio-invasive fungal infection. It usually develops in immunocompromised patients and diabetes is a common predisposing factor [2]. Despite aggressive surgical debridement and antifungal therapy, the prognosis of disease is markedly poor. Antifungal agents have poor tissue penetration property so surgical intervention should be considered first for the treatment of invasive fungal infection. Due to need for extensive surgical debridement, role of other treatment modalities should be explored. Here, we describe three patients with ROCM with varied clinical stages, treated with conventional therapy i.e. amphotericin B later posaconazole was started for follow up.

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Case report

A 50 year old male patient who had uncontrolled type 2 diabetes mellitus for one and a half years presented with high fever for 10 days along with pain and swelling in left side of face and eye. Later, patient developed diplopia and unilateral decrease in vision for five days followed by complete loss of vision for last two days. It was accompanied by difficulty in swallowing specially for solids, non bilious vomiting and change in voice. General physical examination was normal. At the time of examination, patient was conscious with oral temperature of 39.8°C, a blood pressure of 140/90 mm Hg and a pulse of 116/min. Vision and ocular movements in the right eye was normal but the left eye showed negative perception of light. Pupils were fixed and dilated. Ophthalmoplegia was present. Movements were restricted in all directions. Initial assessment showed leucocytosis with raised ESR and slightly decreaased kidney function test. The initial HbA_{1c} was 13.6% on oral hypoglycemic regimen. Chest X ray was normal. ENT examination showed blackish

ulcerating growth in middle meatus. Potassium hydroxide mount (KOH) proved mucormycosis and later contrast enhanced MRI revealed left rhino-orbito-cerebral mucormycosis with cavernous sinus thrombosis. Involvement of left sided ethmoid, maxillary and frontal sinus was also present. There was intraorbital extension into medial orbit, medial rectus, orbital apex, inferior orbital fissure with slight extension into infratemporal fossa. Left cavernous sinus appeared bulky. Patient was started on liposomal amphotericin B along with broad spectrum antibiotics. Diabetes was controlled with multi dosage insulin regimen (on a dose of approx 0.9 units per kg body weight). Amphotericin B was started at a dose of 3 mg/kg which was escalated to 5 mg/kg and continued for 6 weeks. No surgical intervention was done. Repeated ENT and ophthalmological consultation was sought. Repeat MRI was done after 6 weeks of injectable therapy that showed no further progression although there was reduced bulkiness in the cavernous sinus. Clinically, ophthlmoplegia and restriction of eye ball movement completely recovered with slight improvement in vision over the time. Patient was put on oral posaconazole therapy at a dose of 800 mg per day and 4 months down the line, patient showed clinical improvement with no signs of radiological worsening.

Another patient, 50 years old diabetic female presented with painful swelling in the left retro-orbital region and nose for five days along with fever and progressive breathing difficulty. On admission, the patient was conscious with oral temperature of 39.4°C, a blood pressure of 120/80 mm Hg and a pulse of 130/min. She was unable to open the left eye and her vision and left pupillary light reflex were lost. There was leucocytosis, ketonemia with normal liver function, renal function and electrolytes. Blood gasometry confirmed metabolic acidosis. The initial HbA_{1c} was 12.9%. Patient was managed as per diabetic ketoacidosis (DKA) protocol and ENT consultation was sought which was suggestive of extensive black necrotic tissue in the nasal cavity. The patient's nasal structure was completely destroyed and the hard palate was perforated. KOH mount was suggestive of mucormycosis. Patient was started on liposomal amphotericin B along with broad spectrum antibiotics. Liposomal amphotericin B was started at a dose of 3 mg/kg which was escalated to 5 mg/kg and continued for 8 weeks. Diabetes was initially controlled with multi dosage insulin regimen (on a dose of approx 1.2 units per kg body weight) and later stabilized on approx 0.75 units per kg body weight. Extensive surgical debridement was done. Patient improved clinically and was discharged with no active residual apparent disease. On follow up, her clinical status remained fair. However, this patient died in the emergency room due to severe pneumonia, respiratory distress with septic shock after one and half years.

The third patient, a 50 years old female with type 2 diabetes for 10 years presented with painful swelling of the right side of face and right eye for one and a half months. On admission, the patient was conscious with oral temperature of 39.4°C, a blood pressure of 130/80 mm Hg and a pulse of 100/min. She was unable to open the right eye although vision and right pupillary light reflex were normal. There was leucocytosis with normal liver function, renal function and electrolytes. The initial HbA_{1c} was 13.4%. ENT consultation was sought that showed extensive fungal sinus invasion. She was started on basal bolus insulin regimen at a dose of 0.5 units per kilogram body weight but control was finally achieved on 0.7 units per kilogram body weight. Liposomal amphotericin B was started at a dose of 3 mg/kg which was escalated to 5 mg/kg and continued for 6 weeks. Surgery was deferred due to extensive intracerebral involvement. As per ENT opinion patient had been put on amphotericin nasal douche. Later, patient was put on oral posaconazole therapy at a dose of 800 mg per day.

Discussion

Mucormycosis is a fatal opportunistic infection caused by fungi *Mucorales, Rhizopus, Mucor* and *Rhizomucor* species. It usually affects immune-compromised or immune suppressed patients [3]. Till date, surgical debridement is the gold standard for the treatment of ROCM. Surgical debridement has its own limitations. There are several issues in the management of ROCM which still remain unclear. Firstly, most of the recommendations advocate surgical debridement as important lifesaving procedure [4]. This option is often limited due to various reasons (extensive and disfiguring surgery). Secondly, there is no consensus about the duration of the amphotericin B treatment despite unanimity that this is always the first line of treatment. There is not enough data to clarify its duration of use and appropriate time of stopping the treatment. Most recommendations mention amphotericin B should be given till there is radiological and clinical improvement [4]. However, it is often seen in clinical practice that radiological recovery beyond a point is minimal and slow in the absence of surgical debridement. This makes it difficult to decide precisely when to stop amphotericin B.

We gave amphotericin B for 6–10 weeks after diagnosis of the patients which was followed by clinical improvement with some radiological recovery (definitely

no worsening). Long term follow up treatment with oral posaconazole or other oral antifungal agents following long use of injectable AMB is not indicated. Yet we tried oral posaconazole in two patients for six to eight weeks. Now the patients are clinically improved with no definite radiological worsening. This suggests that posaconazole may be useful as a step down therapy after initial amphotericin B treatment. This case report suggests that opportunistic infections are commonly associated with poorly controlled diabetes. Rhino--orbito-cerebral mucormycosis appear to be one of the primary infection among opportunistic infections.

So, the alternative efficacious therapies are being explored. A few experimental reports have shown promising results with posaconazole or ravuconazole but clinical data shows little or no effect [5]. According to a case report, posaconazole with amphotericin B has shown some promising result [6]. In addition to posaconazole, isavuconazole has been approved by FDA [7].

Conclusion

This data supports the essential and alternative treatment modalities for the treatment of rhino-orbito--cerebral mucormycosis. Oral antifungal agents e.g., posaconazole appears to have promising role as add on therapy in treatment of ROCM.

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CASE REPORT
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Levofloxacin-induced life-threatening hypoglycemia in a type 2 diabetic patient with ST-segment elevation myocardial infarction and community-acquired pneumonia

ABSTRACT

Levofloxacin is a broad-spectrum, third-generation fluoroquinolone antibiotic used in the treatment of respiratory and urinary tract infections. Although it is usually well-tolerated, it may cause life-threatening adverse effects, including severe hypoglycemia. We present a case of levofloxacin-induced life-threatening hypoglycemia in a 87-year-old type 2 diabetic patient with ST-segment elevation myocardial infarction and community-acquired pneumonia. Hypoglycemia secondary to levofloxacin is a rare complication (< 0,1%), but can be more common among elderly patients, with type 2 diabetes (especially treated with hypoglycemic drugs) or renal dysfunction. Our patient was at high risk due to age, diabetes and chronic kidney disease (creatinine 149 μmol/L, estimated glomerular filtration rate 27 mL/min/1.73 m²). In the Naranjo probability scale, the patient scored 5 points, which indicates that hypoglycemia was a probable levofloxacin-related adverse effect. In conclusion, we suggest that levofloxacin should be used with greater caution, particularly in patients at increased risk of hypoglycemia. (Clin Diabetol 2020; 9; 2: 141–143)

Key words: type 2 diabetes mellitus, ST-segment elevation myocardial infarction, hypoglycemia, levofloxacin, fluoroquinolone

Introduction

Levofloxacin is a broad-spectrum, third-generation fluoroquinolone antibiotic used in the treatment of respiratory and urinary tract infections [1–3]. Although it is usually well-tolerated, it may cause life-threatening adverse effects, including severe hypoglycemia [2–7].

Case presentation

An 87-year-old woman, a heavy smoker, with well-controlled type 2 diabetes (treated with glimepiride 2 mg/d), chronic kidney disease, arterial hypertension, hyperlipidemia, hyperthyroidism (treated with methimazole 20 mg/d), with frailty syndrome and chronic obstructive pulmonary disease, was admitted to the hospital due to acute anterior ST-segment elevation myocardial infarction (STEMI). On admission, the glycated hemoglobin (HbA_{1c}) level was 5.5% (37 mmol/mol), circulating thyrotropin (TSH) was 0.802 (normal: 0.270–4.200) μIU/mL, free triiodothyronine (fT3) was 4.34 (normal: 3.10–6.80) pmol/L and free thyroxine (fT4) was 15.95 (normal: 12.00–22.00) pmol/L. Serum creatinine concentration and estimated glomerular filtration rate (eGFR) were 168 μmol/L and 23 ml/

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Table 1. Naranjo Adverse Drug Reaction (ADR) Probability Scale [2]

Question	Yes	No	Do not know	Score in the presented patient
Are there previous conclusive reports on this reaction?	+1	0	0	**+1**
Did the adverse event appear after the suspected drug was administered?	+2	–1	0	**+2**
Did the adverse reaction improve when the drug was discontinued or a specific antagonist was administered?	+1	0	0	**+1**
Did the adverse reaction reappear when the drug was readministered?	+2	–1	0	**0**
Are there alternative causes (other than the drug) that could on their own have caused the reaction?	–1	+2	0	**0**
Did the reaction reappear when a placebo was given?	–1	+1	0	**0**
Was the drug detected in the blood (or other fluids) in concentrations known to be toxic?	+1	0	0	**0**
Was the reaction more severe when the dose was increased, or less severe when the dose was decreased?	+1	0	0	**0**
Did the patient have a similar reaction to the same or similar drugs in any previous exposure?	+1	0	0	**0**
Was the adverse event confirmed by any objective evidence?	+1	0	0	**+1**
				Total score: 5

Scoring: ≥ 9 = definite ADR; 5–8 = probable ADR; 1–4 = possible ADR; 0 = doubtful ADR

/min/1.73 m^2, respectively. Fasting blood glucose levels were between 80 and 140 mg/dL and less than 180 mg/dL two hours after meal.

Urgent coronary angiography revealed subtotal proximal left anterior descending coronary artery stenosis and primary percutaneous angioplasty with drug-eluting stent implantation was immediately performed. On admission, the patient was diagnosed with community-acquired pneumonia and antibiotic treatment with ceftriaxone was initiated. On hospital day 6th, ceftriaxone was replaced with levofloxacin (500 mg intravenously once daily) due to the lack of response to the treatment. After three doses of levofloxacin, on the 8th day of hospitalization, the patient developed symptoms of neuroglycopenia with decreased capillary blood glucose to 30 mg/dL. There was no deterioration in renal function (creatinine concentration and eGFR were 146 μmol/L and 25 mL/min/1.73 m^2, respectively). Within following 8 hours, seven ampoules of 10 ml 40% glucose injection and 1000 ml of 5% glucose infusion were administered. Then, due to persistent tendency to hypoglycemia, a continuous intravenous infusion of 40% glucose at 10–50 mL per hour was begun. Glimepiride was discontinued. After 5 days of successful antibiotic treatment, levofloxacin was switched from intravenous to oral administration and then discontinued. Gradual normalization of glycemia was observed. On the 11th hospital day intravenous glucose infusion was stopped. No further episodes of hypoglycemia were observed during the next days of hospitalization. After recovery, the treatment of diabetes was changed. Based on the current recommendation of Polish Diabetes Association and due to chronic kidney disease with final eGFR of 27 mL/min/1.73 m^2 and persistently increased risk of hypoglycemia the therapy with glimepiride was changed to linagliptin 5 mg/d.

Discussion

Levofloxacin is considered as a high effective and relatively safe fluoroquinolone antimicrobial used in the treatment of community-acquired pneumonia, complicated urinary tract infections and acute bacterial sinusitis [1–3]. Hypoglycemia secondary to levofloxacin is a rare complication (< 0,1%), but can be more common among elderly patients, with type 2 diabetes (especially treated with hypoglycemic drugs) or renal dysfunction [2]. Our patient was at high risk due to age, diabetes and chronic kidney disease. In the Naranjo probability scale, the patient scored 5 points, which indicates that hypoglycemia was a probable levofloxacin-related adverse effect (Tab. 1) [8].

The mechanisms underlying levofloxacin-induced hypoglycemia in diabetic patients are not fully understood. There is increasing evidence that they are related to drug-drug interactions [9] or hyperinsulinemia [10]. It is postulated that there are two main pathomechanisms that may lead to fluoroquinolone-induced hypoglycemia: pharmacokinetic and pharmacodynamic [9–12].

The first one is associated with competitive metabolism of levofloxacin and sulfonylureas by cytochrome P450 2C9 (CYP2C9) [9–12]. Pharmacodynamic theory is based on presumed fluoroquinolone-induced increase in insulin release from pancreatic beta-cells [11]. Levofloxacin inhibits ATP-sensitive potassium channels causing depolarization of the beta-cell membrane, which results in opening voltage-dependent calcium channels and insulin secretion [11]. Other experimental studies using rat islet cells suggest that fluoroquinolones act not as initiators but rather as augmenters of stimulated insulin release from beta-cells [12]. Of the three fluoroquinolones examined in the study with mouse pancreatic islets, levofloxacin appeared to be less likely to cause hypoglycemia than temafloxacin and gatifloxacin (both withdrawn from the market because of side effects, including hypoglycemia) [11]. However, in another study on rats, levofloxacin significantly affected glucose serum concentration in a dose-dependent manner [13].

Awareness about levofloxacin-induced hypoglycemia among physicians is relatively low. In a survey conducted in one of the university-affiliated teaching hospital, 80.4% of respondents were unaware about this adverse effect [4]. Importantly, this result may be underestimated due to a fatal case of levofloxacin-related hypoglycemia in this hospital, shortly before the questionnaire was conducted [4].

Clinical conditions to consider in the differential diagnosis of hypoglycemia in our patient include the following: chronic kidney disease, malnutrition, hypoglycemic effect of glimepiride, and less frequently — hyperthyroidism.

The patient had a long history of chronic kidney disease, with no episodes of hypoglycemia in the past. We also found no deterioration in renal function during hospitalization. Another cause of hypoglycemia might be malnutrition. However, during the patient's stay in hospital, her nutritional status was good and she weighed approximately 60 kilograms. Glimepiride, which was used by the patient, may also induce hypoglycemia, however we have no reports of hypoglycemia episodes in the past medical history of the patient. In addition, despite the use of glimepiride during hospitalization, blood glucose levels were normal until levofloxacin was included. Finally, hypoglycemia may have been a rare but possible complication of hyperthyroidism. It is well known that increased thyroid hormone levels are conducive to carbohydrate metabolism disorders. Importantly, hyperthyroidism usually predisposes to hyperglycemia, due to intensified hepatic gluconeogenesis, insulin resistance and increased glucose absorption from the gastrointestinal tract. An additional argument against hyperthyroidism-induced hypoglycemia is that the patient was in euthyroid state during hospitalization.

In conclusion, we suggest that levofloxacin should be used with greater caution, particularly in patients at increased risk of hypoglycemia.

Conflict of interest

The above-mentioned authors declare that there is no conflict of interest.

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VIA MEDICA
REVIEW ARTICLE
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Coffee in the diet and prevention of diabetes

ABSTRACT

The impact of coffee consumption on health deserves an attention, especially in the context of patients' ongoing concerns about its adverse health effects. Knowledge on this subject may also inform the clinical practice. Objective — to present the current state of knowledge about the relationship between coffee consumption and the incidence of diabetes type 2.

Epidemiological studies indicate that the habit of coffee consumption reduces the risk of non-insulin-dependent diabetes. Drinking at least 3 cups of coffee a day reduces the risk of this disease by 20–25%, and higher consumption may portend even more protection. The beneficial effect of coffee is most likely due to polyphenols present in the infusion, which have an anti-inflammatory effect and may improve insulin sensitivity. The content of bioactive ingredients in a cup of coffee is variable, depending on both the natural variation in coffee beans and the brewing method. The issue of sweetening coffee is a particularly important one as the addition of sugar can reduce its beneficial effects. Moderate consumption of coffee brewed using filters (3–5 cups per day) is recommended.

Current scientific research indicates that coffee not only has no negative impact on health but may even reduce the incidence of diabetes. (Clin Diabetol 2020; 9; 2: 144–148)

Key words: coffee, diabetes, health

Introduction

Diabetes type 2 is one of the most common metabolic diseases, associated with excess body weight, sedentary lifestyle, and inappropriate diet. Nutrition scientists are thus looking for dietary approaches to reduce its incidence. In addition to recommendations to reduce sugar intake and increase intake of vegetables and whole grain products, dietary guidelines have included a recommendation to drink coffee [1, 2]. An analysis of the results of recent studies on the health effects of drinking coffee led to including a cup of coffee in the recently updated food pyramid of the Food and Nutrition Institute [3]. Thus, after years of the prevailing belief of negative or at least risky health effects of coffee drinking, currently available studies indicate otherwise. Coffee is no longer considered a substance, which is defined as a product which has no nutritional value for the body, but rather as a component of a healthy diet. According to experts from the United States and the Netherlands, moderate coffee intake fits well within the currently recommended nutrition, and coffee may even be included among so-called functional foods [2, 4–6].

Coffee and its constituents

Similarly to other food products, coffee contains specific compounds. In addition to well-known caffeine, these include polyphenols, diterpenes, niacin, and magnesium.

Caffeine in an alkaloid occurring naturally in some plant sources, mainly coffee, tea, and cocoa beans. Food industry also uses synthetic caffeine, added as an aroma (e.g., in cola-type beverages) or a psychoactive ingredient (e.g., in energy drinks) [7]. Research indicates that the lowest caffeine dose which induces measurable physiological effects in the human body is 75 mg. A single dose of 75 mg increases alertness and attention in most adults but its effect may be variable and depends on genetic and environmental factors. It has no effects in some subjects, termed "non-responders

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to caffeine" [8, 9]. Safe daily intake of caffeine in adults is up to 400 mg, and up to 2.5 mg/kg body mass in children. Excessive caffeine intake may lead to adverse effects including hyperexcitability, muscle tremor, insomnia, headache, and gastric upset [9]. According to the International Statistical Classification of Diseases and Related Health Problems, negative effects of large caffeine intake are categorized as mental and behavioural disorders due to use of other stimulants including caffeine (code F 15.3) [10]. The main action of caffeine in the body is by blocking the receptor for adenosine, a brain neurotransmitter inducing the feeling of fatigue, which leads to increased catecholamine levels (adrenaline, dopamine, serotonin) resulting in the central nervous system stimulation, increase in heart rate, and vasodilatation [11, 12]. This mechanism leads to an overall improvement of mental and physical performance and thus caffeine has an ergogenic effect [13, 14]. Studies performed in the recent years failed to confirm the previous hypothesis of a beneficial effect of caffeine on fat burning. Thus, nutritional law regulations prohibit suggesting weight-loss properties of caffeine, e.g., on dietary supplements product packages [15].

The caffeine content in coffee is very variable and depends on multiple factors including the botanical species of coffee (Robusta contains twice more caffeine compared to Arabica), cultivation conditions, the degree of bean crushing, brewing method, and the strength of infusion. Due to these factors, it is difficult to predict caffeine content in an individual cup of coffee. Literature data indicate that a cup of coffee may contain 28 to 322 mg of caffeine [16–19]. In one study, the caffeine content in 20 espresso coffees purchased in various cafes varied up to 6-fold [18]. In general, it has been estimated that the average caffeine content in a typical cup of coffee is 80–90 mg [20].

Coffee is also a source of polyphenols. These are a group of plant compounds with complex chemical structure, of which the best known are flavonoids present, e.g., in fruits, tea, and dark chocolate. Polyphenols are food compounds with anti-inflammatory and antioxidant properties. Their large intake is believed to exert an effect comparable to that of non-steroidal anti-inflammatory drugs [21]. Similarly to caffeine content, their content in coffee infusion is very variable. It has been estimated that a cup of coffee contains on average about 190 mg of polyphenols [22]. For a comparison, 100 g of grapes provide 200–300 mg of polyphenols [23]. However, it is difficult to evaluate these amounts in terms of health benefits as the recommended polyphenol intake has not been established. The major polyphenol in coffee is chlorogenic acid which is also considered the most biologically active [24]. Due to its breakdown during prolonged heat processing, the largest amounts of chlorogenic acid are present in green (unroasted) coffee beans [5].

Among other coffee compounds, of major importance are diterpenes (lipid compounds) which may have both positive and negative health effects. While coffee with a high diterpene content increases LDL cholesterol, triglyceride, and homocysteine levels, these compounds also activate liver enzymes which facilitate elimination of toxic metabolism waste products. Diterpenes are most abundant in unfiltered coffee (e.g., Turkish coffee) but nearly absent in filtered and instant coffee [2, 25]. Coffee infusion also contains niacin and magnesium. According to the Polish national data, a cup of coffee provides 1 mg of niacin and 19 mg of magnesium, or 5–6% of their recommended daily allowance [26].

Coffee in the prevention of diabetes

Potential effect of coffee on reducing the risk of non-insulin-dependent diabetes has been discussed in the scientific literature for several years. The interest in this issue surged in 2002 when Dutch researchers published a large epidemiological study that included a population of more than 17 000 and showed a lower incidence of diabetes among regular coffee drinkers [27]. The most pronounced effect was noted for the intake of 7 cups per day, with a twice lower incidence of diabetes compared to non-drinkers and those consuming less than 2 cups per day. Consuming 5–6 cups per day was associated with a 27% reduction in diabetes incidence, and the lowest effect was seen for the consumption of 3–4 cups per day (21% reduction in diabetes incidence). In the subsequent years, two independent studies performed in the Netherlands and Japan showed that consuming at least 3 cups of coffee per day was associated with a 42% reduction in diabetes incidence [28, 29]. Somewhat lower reduction in risk (by 27%) was noted among French women consuming 3 cups of coffee per day [30].

Studies performed in the United States indicate that the effect of coffee consumption on the development of diabetes may be gender-related. Men drinking at least 6 cups of coffee per day had a 54% lower risk of diabetes, while women at the same level of coffee consumption had a 22–29% lower risk [31, 32]. However, different results were obtained in a study in Finland, in which a very high coffee consumption (at least 10 cups per day) was associated with diabetes incidence reduction by 55% in men and 79% in women [33]. No studies have been performed to evaluate the effect of coffee consumption on the risk of diabetes type 2 in Poland.

Metaanalyses of research studies, which provide an objective summary of the available knowledge, have also provided optimistic conclusions in this regard for years. The first of these, published in 2005, showed that people usually consuming 4–6 cups of coffee per day had a 28% lower incidence of diabetes compared to non-drinkers and those consuming less than 2 cups per day, and those consuming more than 6 cups of coffee per day had a 35% lower incidence of diabetes [34]. A subsequent 2009 metaanalysis showed a linear relationship between the amount of coffee consumed and diabetes incidence. The consumption of 3–4 cups of coffee per day was associated with a 25% reduction in diabetes incidence, and each additional cup of coffee reduced this risk by further 7% [35]. A metaanalysis published in 2014 showed that consuming 3 cups of coffee per day was associated with a 21% reduction in diabetes incidence, and consuming 6 cups of coffee per day was associated with a 33% reduction in diabetes incidence [36]. Very similar conclusions were arrived in the most recent 2018 metaanalysis which showed that consuming 3 cups of coffee per day was associated with a 21% reduction in diabetes incidence, and consuming 5 cups of coffee per day was associated with a 29% reduction in diabetes incidence [37].

Of note, most studies available in the literature are observational epidemiological studies and not randomized clinical trials. Such studies may not account for all risk factors for diabetes, and thus the effect attributed to coffee consumption cannot be considered proven [38]. One argument against the prophylactic effect of coffee is the number of patients with diabetes in countries with a very high coffee consumption, such as Sweden and the Netherlands. According to the 2013 data, the prevalence of diabetes in these countries was 6.4% and 7.5%, respectively, which is similar to the prevalence in Poland (6.5%), where drinking coffee is much less common [39]. A hypothesis of the effect of coffee consumption on decreasing the risk of diabetes has been questioned by the results of a multicenter Mendelian randomization study which failed to show an association between coffee consumption and diabetes incidence [38].

Potential mechanisms of the effect of coffee on the prevention of diabetes

The mechanism of the effect of coffee on the risk of diabetes has not been elucidated. Short-term effects of acute coffee ingestion in individuals who do not drink it regularly are negative and include reduced insulin sensitivity and impaired glucose tolerance. In regular coffee drinkers, likely due to development of tolerance, insulin sensitivity increases and blood glucose level is lowered [32, 40]. Caffeine itself is not considered a putative mediator of this effect, as studies showed beneficial effects also for caffeine-free coffee [36, 37]. Most experts suspect that anti-inflammatory properties of coffee polyphenols may be of key importance [41]. Coffee consumption may reduce levels of proinflammatory cytokines, considered a risk factor for diabetes [42], and chlorogenic acid may inhibit hydrolysis of glucose-6-phosphate to glucose and reduce glucose absorption in the gastrointestinal tract [30, 43].

When considering the health effects of coffee consumption, the variable mode of brewing coffee should not be overlooked. Of note, coffee automatic machines are gaining popularity, and the health effects of coffee brewed this way have not been evaluated yet. Although experts believe that the ideal coffee brewing method in terms of its nutritional and health effects has not been defined, filtered coffee is most recommended [2, 44, 45]. It is also clear that addition of large amount of sugar or cream increases overall dietary content of these products, which may counteract positive health effects of coffee compounds. When adding two teaspoons of sugar to a cup of coffee, 3 cups of coffee, which are a considered a minimum amount associated with a reduction in diabetes incidence, provide 60% of recommended maximum daily intake of added sugar (which includes both sugar added by the consumers themselves, e.g. sweetened coffee, and sugar found in processed food). Furthermore, adding large amounts of milk may reduce the beneficial effect of polyphenols by their binding to milk protein, resulting in formation of insoluble and biologically inactive polyphenol complexes [46]. The contents of major compounds in a cup of coffee depends on the strength of the infusion. Stronger coffee contains more polyphenols but also more psychoactive caffeine. Another confounding factor is the size of a cup of coffee that is used a measure of coffee consumption. In the United Stated, a typical portion of coffee is about 250 mL, while in Europe it is much smaller (125–160 mL). A cup of espresso coffee which has recently become popular contains only 25–40 mL [47–49].

Summary

Despite varying patterns of coffee consumption worldwide, studies from many research centers have yielded positive findings for coffee, supporting the hypothesis of its health benefits. Despite these optimistic data, which also suggest other benefits of coffee consumption in addition to diabetes prevention, including its potential anti-cancer effect, up to 75% of consumers still believe that coffee has a negative impact on health [2, 11]. Thus, education efforts should be

pursued regarding the safety of coffee consumption, so the patients will no longer worry about its negative health effects. If future findings substantiate current hypotheses, coffee lovers will include not only those who enjoy its taste but also those who will appreciate its positive health effects.

Conflict of interest

The author declares no conflicts of interests.

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