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Exocrine pancreatic insufficiency related fat malabsorption and its association with autonomic neuropathy in Asian Indians with type 2 diabetes mellitus

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ABSTRACT

Background and aims: We aimed to estimate the prevalence of exocrine pancreatic insufficiency (EPI) related fat malabsorption & to correlate it with measures of autonomic neuropathy in patients with T2DM from India.
Methods: Patients with T2DM (cases; n = 118) and normo-glycaemic individuals (controls; n = 82) underwent anthropometry and biochemical evaluation at baseline. The 72-hours fecal fat excretion was estimated by the Van de Kamer's titration method. Autonomic neuropathy was evaluated using an automated analyzer.
Results: The prevalence of EPI related fat malabsorption in cases was 45% (n = 53; 72 hours mean fecal fat level = 22.7 ± 5.6 g/day). Dysfunctions in the parasympathetic nervous system (PNS; 86.7%; $p < 0.05$), sympathetic nervous system (SNS; 92.4%; $p < 0.05$), and both; PNS + SNS (83.1%; $p < 0.05$) were significant. Amongst measures of PNS dysfunction, there was a significantly higher percentage of abnormal expiration: inspiration ratio (45.3%) and the 30:15 ratio (84.9%) ($p < 0.05$) in patients with T2DM and EPI related fat malabsorption.
Conclusion: In this cross-sectional cohort of Asian Indian patients with T2DM (n = 118), EPI related fat malabsorption correlates significantly with autonomic dysfunction in patients with T2DM. However, these preliminary data need to confirmed in trials with more robust design.

1. Introduction

An exponential increase in the prevalence of type 2 diabetes mellitus (T2DM) is evident with an estimate of 7079 affected individuals per 100,000 globally by the next decade [1]. Diabetes is an endocrine disorder due to derangements in the exocrine and endocrine functions of the pancreas [2]. The endocrine and exocrine pancreas are closely related and exocrine pancreatic insufficiency (EPI) is primarily related to insufficient enzymatic secretion from the acinar cells of the pancreas (3). EPI can cause fat malabsorption, steatorrhea, weight loss and gastrointestinal discomfort in patients with T2DM [4]. Conventionally, it had been opined that patients with T2DM have normal exocrine pancreatic function. However, recent studies have shown some evidence that may be in variance from conventional thinking [5,6].

A close association between cardiovascular status and autonomic dysfunction exists in patients with diabetes mellitus [7,8]. Autonomic neuropathy and microvascular damage in patients with T2DM may play a role in the occurrence of EPI, in the absence of autoimmune damage and insulin deficiency [9]. Physiologically, the exocrine pancreatic response to a meal intake is mediated by entero-pancreatic reflexes that are regulated by peptides and neurotransmitters from the gut, the pancreas and vagal stimulation [10]. Therefore, disruption of entero-pancreatic reflexes by autonomic neuropathy may impair exocrine pancreatic function [11]. Steatorrhea with fecal fat levels above 7 gms/day is suggestive of EPI related fat malabsorption [12], thereby leading to a poor quality

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of life in patients with long standing, uncontrolled diabetes mellitus. Notably, studies have shown that patients with diabetes mellitus and EPI have a high morbidity and mortality risk, secondary to malnutrition-related complications, autonomic and peripheral neuropathy [13].

The clinical diagnosis of EPI can be made using direct or indirect pancreatic function tests, and should ideally be done before the onset of complications. Direct tests of pancreatic function include measuring bicarbonates, enzyme concentrations and pancreatic fluid secretions, in response to intravenous stimulation of the pancreas by hormones such as secretin, cholecystokinin, or a combination. These invasive tests are accurate for the diagnosis of mild EPI but are limited in utility due to high costs.

On the other hand, indirect tests do not involve hormonal stimulation and are applicable for the diagnosis of moderate to severe EPI. One of such tests is the ^{13}C mixed triglyceride breath test (MTBT) which measures intra-luminal lipolytic activity as a measure of exocrine pancreatic function. It involves ingestion of a standardized meal containing tracer-labelled triglycerides and measurement of the exhaled air at 15-min intervals for 6 hours, to compare the amount of exhaled ^{13}C to the actual ingested dose. A decreased recovery of ^{13}C indicates impaired pancreatic lipase secretion. Despite its high accuracy value, the MTBT is not applicable as it requires the patient to be in a restrained seated position for continuous 6 hours to reduce interference of physical activity with endogenous carbon dioxide production [14].

Another indirect test for malabsorption is the ^{13}C-D xylose breath test. In this test, one gm of ^{13}C-D xylose dissolved in 500 ml of water is given for oral consumption, after an overnight fast. This is followed by CO_2 sampling from exhaled air done every 30 minutes for a total of 3.5 hours [15]. However, despite optimal sensitivity, this test is limited in utility due to low specificity and rigorous sampling method.

Amongst indirect tests, the gold standard for measuring mild to moderate EPI associated malabsorption is the quantitative estimation of 72 hours fecal fat [16,17]. However, this procedure is cumbersome and unpleasant as the patient is required to follow a strict diet, with dietary fat intake of 50 g per day for five consecutive days and to collect the whole volume of feces for the last three days/(terminal 72 h). On the other hand, laboratory personnel need to homogenize large volumes of fecal samples for the assay. Owing to the laborious techniques involved, this test is performed only in a few specialized centers, primarily for research purposes. Therefore, as an alternative strategy, the ELISA based estimation of fecal elastase-1(FE-1) is widely utilized in diagnostic laboratory, as it is more rapid and simple to perform with a high sample turnover rate. Although FE-1 is a sensitive test in the diagnosis of EPI, it has a lower specificity due to cross reactivity with antigens differing from elastase-1, leading to false positive reports in FE-1 ELISA have also been noted [18,19]. A previous study in Asian Indians has shown that despite high sensitivity (85%) of the FE-1 assay, it had demerits of low specificity and lack of correlation with the 72 hour mean fecal fat values, suggesting that the FE-1 assay cannot be used independent of the 72 hour fecal fat assay [20] A meta-analysis had shown that, despite robust sensitivity of the FE-1, assay, a false-negative rate of 1.1% and a false-positive rate of 11%, is possible in patients with fecal elastase-1 levels with less than 200 mcg/g of feces, thereby indicating low specificity [21]. Furthermore, liquified fecal samples may also produce a false positive result in the FE-1 ELISA [22,23]. In comparison to the FE-1 ELISA, the results of the fecal fat assay are highly reproducible as fecal fat is stable in solid feces for up to one week at room temperature, and upto one month when stored at four degrees Celsius [24]. Despite its limited utility, the 72-hour fecal fat assay assumes significance for the precise diagnosis of mild to severe EPI [25].

The prevalence of EPI is variable, and much of the symptoms in the earlier stages of its onset may be mild or under reported by patients with T2DM [26]. Most studies on the prevalence of EPI are based on FE-1, while those based on 72-hours fecal fat estimation are scarce, especially in Asian Indians. We hypothesized that high prevalence of exocrine insufficiency related fat mal-absorption would be related to reduced parasympathetic activity in patients with T2DM. Therefore, the primary objective of this study was to estimate the prevalence of EPI related fat malabsorption in patients with T2DM using the 72-hours fecal fat assay and the secondary objective was to study the association of EPI related fat malabsorption with measures of autonomic dysfunction.

2. Methodology

2.1. Study design

The study protocol was reviewed and approved by the Institutional Review Board (IRB) for ethics in research involving humans (IRB min No. 11119 [DIAGNO] dated January 10, 2018. This cross-sectional study was conducted in the Department of Endocrinology, Diabetes and Metabolism, CMC, Vellore, India, over a period of 12 months between the years 2018−2019. Patients with T2DM, aged between 40 and 70 years with a duration of the disorder lasting for at least one year from the point of diagnosis were included in the study with informed, written consent. As for the controls, age and BMI matched normoglycemic subjects from the local community, aged between 40 and 60 years, with no history of systemic illness, gastro intestinal disorders, systemic illness, acute or chronic pancreatitis, pancreatic malignancies, pancreatic or gastrointestinal surgeries, alcohol intake, or substance abuse and willing to participate in the study were recruited. Subjects with known type 1 diabetes mellitus, pregnancy, clinical or documented evidence of chronic pancreatitis, ductal dilatation or intra-ductal calculi on imaging, malignancy, co-existent systemic infections, end-stage renal disease, chronic alcohol dependence, pancreatic enzyme supplement intake, alcohol induced pancreatitis were excluded from the study. Furthermore, patients with history of cerebrovascular diseases, active cardiovascular disease, lower limb amputations, psychiatric illness were also excluded from participation.

2.2. Sample size calculation

With a previously reported prevalence rate of EPI of about 29% in Asian Indians [27], the odds of developing exocrine insufficiency in patients with T2DM was expected to be five times higher. The sample size was calculated as a total of 200 subjects comprising of 118 patients with T2DM (cases) and 82 non-diabetic, healthy individuals (controls) with a power of 80% (alpha error: 5% & beta error: 20%) at 95% Confidence interval (CI).

2.3. Baseline assessment, anthropometry and 72-h fecal fat assay

All subjects (cases and controls) underwent anthropometric assessment for body mass index (BMI) and waist circumference. Baseline biochemical investigations (fasting and postprandial blood glucose levels, Hba1c, serum creatinine, fasting serum lipid profile) were done. History of medications was obtained from the electronic medical records of the institution.

For the 72-hours fecal fat estimation, T2DM patients (cases; $n = 118$) and normo-glycaemic subjects (controls; $n = 82$) were provided with 50 g of butter daily along with a predefined meal for five days thereby ensuring dietary intake of at least one hundred grams of fat per day throughout the study period. They were

contacted daily over telephone to ensure compliance with dietary fat intake and to encourage sample collection. After the first two days of fat loading, the patients were instructed to collect whole volumes of fecal samples in labelled, air tight sample containers for three consecutive days and deposit the samples at the cold storage facility of the laboratory without delay, and passing the information to the laboratory staff.

The average fat volume in a 72-h fecal sample was estimated using the titration method of van de Kamer [28]. Briefly, 5 g of homogenized fecal sample was emulsified with 40 ml of 95% ethyl alcohol containing 0.4% amyl alcohol, and 10 mL of 33% potassium hydroxide, which was further treated with 17 mL of 25% hydrochloric acid. Fifty ml of petroleum ether was then added for separation of the ether-fat from water layers. Twenty-five ml of the supernatant was removed from the ether-fat layer and evaporated to dryness on a hot plate. Ten milliliters of neutral alcohol were added to the dried fecal fat and the solution was titrated with 0.1 N sodium hydroxide. The volume of fatty acid per 100 g of stool were calculated from the milliliters of sodium hydroxide necessary for titration and the average molecular weight of dietary fatty acid. This expression of the fecal fat concentration was converted to a daily output value by multiplying the mean daily fecal weight [28]. EPI was diagnosed as >18 g fat in the 72-h fecal sample. T2DM patients were categorized into those without exocrine pancreatic insufficiency and those with exocrine pancreatic insufficiency and parameters of cardiac autonomic function were compared. The presence of neuropathy in the lower limbs of T2DM patients was recorded through an oral interview. Peripheral neuropathy was assessed using a 10 g monofilament and vibratory perception using a biothesiometer (Diabetik foot care, India, Pvt Ltd®) Diabetic retinopathy was diagnosed on the basis of fundus photography or history of laser therapy Nephropathy was diagnosed when albuminuria was >30 mg over 24 h or when estimated creatinine clearance was <60 ml/min [29].

2.4. Symptomatic assessment of autonomic dysfunction

We performed a subjective assessment for autonomic dysfunction in T2DM patients using the Survey of Autonomic symptoms (SAS) questionnaire, which has been validated earlier in clinical trials as well as in epidemiological studies for the symptomatic diagnosis of early diabetic peripheral neuropathy, independent of confounders such as age, gender, body mass index. The SAS scale assessed the presence of symptoms and the degree of severity. Each item was rated by an impact score ranging from 1 (mild) to 5 (severe) [30]. The SAS questionnaire with 11 and 12 questions for women and men respectively was filled by patients with T2DM ($n = 118$) prior to the objective tests.

2.5. Objective assessment of autonomic nervous dysfunction

Evaluation of autonomic dysfunction was conducted in subjects with T2DM (cases; $n = 118$) using a computerized autonomic function analyzer (CANS 504, Diabetik Foot care Pvt Ltd, India) (Fig. 1), based on heart rate variation (RR interval) measurements during rest, deep breathing, Valsalva maneuver and orthostatic positions. The patients were instructed to avoid smoking, caffeinated beverages and antihypertensive medications for at least 3 hours prior to testing. After a resting period of 5 minutes in supine position, the blood pressure and electrocardiograms (ECG) were measured in gentle breathing and in deep breathing status. This was followed by blood pressure and ECG assessment in the standing position. A compressible metallic hand grip was provided to the patient to compress and retain up to one's threshold strength in a span of 60–120 seconds. The blood pressure and ECG patterns in response to exertion were recorded. An abnormal blood pressure in response to standing and/or sustained hand grips indicated sympathetic nervous dysfunction. This was followed by the Valsalva maneuver wherein the patient was instructed to inhale deeply and exhale with exertion into a connected external mouth piece. The tests of the parasympathetic nervous system (PNS) included the following: a) the heart rate response to deep breathing expressed as expiration: inspiration (E:I) ratio, b) the heart rate response to Valsalva maneuver (exertion) expressed as Valsalva ratio and c) the heart rate response to standing expressed as 30:15 ratio. The data were processed by standard, vector and spectral analysis through an in-built algorithm of the CANS 504 machine. The results obtained were compared to normal values that were adjusted for age. Parasympathetic nervous system (PNS) dysfunction was diagnosed if any one or more of the three tests (the E:I ratio, the Valsalva ratio, 30:15 ratio) were found to be abnormal. Based on Ewing's criteria [31], autonomic dysfunction was

Fig. 1. The computerized autonomic neuropathy analyser (CANS 504).

Table 1
Comparison of baseline characteristics between groups.

Variables	Patients with T2DM (n = 118)	Normo-glycaemic subjects (n = 82)	*P* value
Age (years)	56.1 ± 8.4	55.8 ± 10.1	0.35
BMI (kg/m^2)	26.4 ± 4.1	25.7 ± 5.3	0.19
Waist circumference (cms)	92.5 ± 11.2	88.3 ± 8.4	0.12
Duration of diabetes mellitus (years)	12.3 ± 6.2	NA	-
HbA1c (gm %)	8.1 ± 1.4	5.2 ± 0.4	< **0.05***
Fasting blood glucose (mg/dL)	156.6 ± 35.4	85.5 ± 8.1	< **0.05***
Post-prandial blood glucose (mg/dL)	238.3 ± 49.2	124.4 ± 22.5	< **0.05***
Serum creatinine (mg/dL)	1.2 ± 0.5	1.1 ± 0.4	0.23
Serum triglycerides (mg/dL)	192 ± 87.3	144 ± 65.8	< **0.05***
Serum LDL-C (mg/dL)	132 ± 58.4	119 ± 50.2	0.11
Subjects with stool fat >18 g	N = 53 (44.9%)	N = 5 (6.1%)	< **0.05**[a]

T2DM = Type 2 Diabetes Mellitus, BMI = Body mass index, LDL-C = Low density lipoprotein cholesterol.
Values are presented as Mean ± SD; *P* value < 0.05: Statistically significant.
[a] Indicates: *P* value of fisher's exact test.

Table 2
Comparison of anthropometric measures and clinical profiles between groups.

Baseline characteristics	Patients with T2DM & without EPI associated fat malabsorption (n = 65)	Patients with T2DM and EPI associated fat malabsorption (n = 53)	
Age (years)	50.8 ± 9.6[a]	53.6 ± 10.5[a]	0.42
Body mass index (kg/m^2)	26.7 ± 3.2[a]	28.5 ± 2.4[a]	0.34
Waist circumference (cm)	90.8 ± 5.6	90.1 ± 4.3	0.42
Duration of Diabetes mellitus (years)	12.3 ± 5.5[a]	10.8 ± 5.9[a]	0.36
HbA1c (%)	8.7 ± 2.2[a]	9.1 ± 3.8[a]	0.25
Prevalence of microvascular/macrovascular complications: n (%)			
Retinopathy	16 (24.6)	10 (18.8)	0.13
Neuropathy	11 (17.6)	23 (43.4)	< **0.05***
Nephropathy	10 (15.4)	11 (20.8)	0.55
Coronary artery disease	8 [12]	6 (11.2)	0.28
Cerebrovascular accident	1 (1.5)	3 (5.8)	0.49
Peripheral arterial occlusive disease	2 (3.1)	1 (1.9)	0.19

P value < 0.05: Statistically significant.
T2DM = Type 2 Diabetes Mellitus.
[a] Indicates mean value with standard deviation.

diagnosed and graded as follows:1) presence of one abnormal finding indicates mild autonomic dysfunction, 2) presence of at least two abnormal findings were confirmative of moderate autonomic dysfunction and 3) presence of orthostatic hypotension in addition to other abnormal findings indicates severe autonomic dysfunction.

2.6. Statistical analysis

Continuous variables were checked for normative distribution and presented as values of Mean ±Standard deviation. Categorical variables were presented as frequency and analyzed by Chi square test or Fisher's exact test as appropriate. Pearson's/Spearman's correlation test was applied to check for significant correlations with the dependent variable and stepwise multiple linear regression analysis were applied to derive significant predictors for the dependent variable. A *p* value < 0.05 was considered as statistically significant.

3. Results

In this study, patients with T2DM (cases; *n* = 118) and normoglycemic individuals (controls; *n* = 82) were matched for age, BMI and waist circumference. As shown in Table 1, the mean value of serum triglycerides and measures of glycaemia were significantly higher in cases, when compared to controls. Amongst cases, EPI related fat malabsorption (72 hours fecal fat >18 g) was detected in 53 subjects (44.9%) while the rest (n = 65; 55%) had no evidence suggestive of exocrine insufficiency. The 72 hours mean fecal fat value in T2DM patients with EPI was 22.7 ± 5.6 g/day. In controls, the EPI was reported only in 5 subjects (6.1%). As shown in Table 2, T2DM patients were categorized based on the absence or presence of fat malabsorption. In T2DM patients without fat malabsorption, the majority of patients (41%) were in the age group of 41–50 years, as compared to those with fat malabsorption (41.5%) wherein most of the patients were in the age group of 51–60 years. Both the groups were predominantly overweight or obese, with a BMI ranging between 25 and 29.9 kg/m^2. The HbA1c levels ranged between 7 and 10% in both the groups and the prevalence of diabetic peripheral neuropathy (*n* = 23/53; 43.4%) was significantly higher (p < 0.05) in patients with T2DM and fat malabsorption compared to patients with T2DM & without fat malabsorption. The patient's medication history is presented in Fig. 2. A higher proportion of patients with T2DM and without EPI related fat malabsorption were on metformin, sulphonylureas and DPP4 inhibitors than those with T2DM and fat malabsorption.

On subjective assessment for symptomatic autonomic dysfunction using the SAS questionnaire, the mean total symptom score (TSS) was significantly higher for T2DM patients with EPI, compared to T2DM patients without exocrine insufficiency (mean TSS: 3.8 ± 1.4: *vs.* 1.6 ± 0.6; p < 0.05). Similarly, the total impact score (TIS) was much higher among those patients with T2DM and EPI (Mean TIS score: 9.0 ± 3.4 *vs.* 5.3 ± 2.1 p < 0.01). The profile of symptoms in patients with T2DM patients with and without EPI

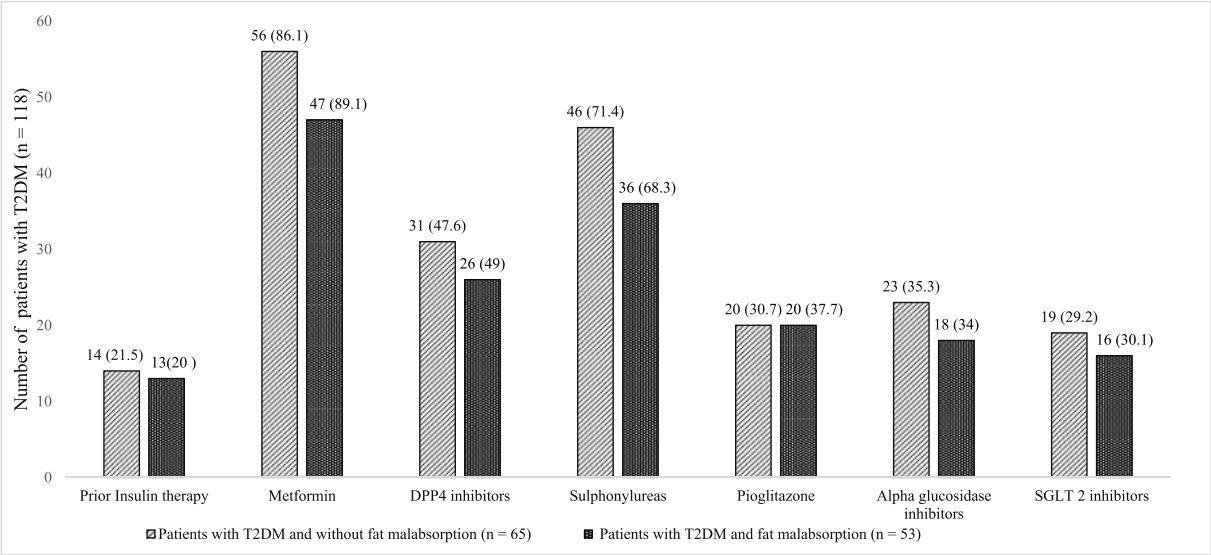

Fig. 2. Profile of medications used in Type 2 Diabetes Mellitus patients with and without Exocrine Pancreatic insufficiency.

related fat malabsorption is shown in Fig. 3. Patients with T2DM and fat malabsorption frequently reported symptoms such as diarrhoea, constipation, and sensation of dry mouth, though it was not statistically significant.

The objective assessment for autonomic neuropathy was performed in all patients with T2DM ($n = 118$) using an automated analyzer (CANS 504). A significantly higher proportion had Parasympathetic Nervous system (PNS) dysfunction (86.7%; $p < 0.05$), sympathetic nervous system (SNS) dysfunction (92.4%; $p < 0.05$), and both the PNS + SNS (83.1%; $p < 0.05$) dysfunctions in patients with T2DM and EPI related fat malabsorption, than those with T2DM and normal exocrine functions. We noted significantly lower heart rate in patients with T2DM and EPI related fat absorption ($p < 0.05$). Amongst measures of the PNS abnormality, significantly higher percentage of abnormal E:I ratio (45.3%) and 30:15 ratio (84.9%) was noted in T2DM patients with EPI related fat malabsorption ($p < 0.05$) (Table 3). A correlation analysis was performed with fecal fat ≥18 g as the outcome variable and age, gender, duration of diabetes, BMI and HbA1c as independent variables. In patients with T2DM and EPI related fat malabsorption, significant positive correlation was observed between the mean stool fat value and duration of diabetes mellitus ($r = 0.58$; $p < 0.05$), whereas a significant negative correlation was noted with HbA1c ($r = -0.65$, $p < 0.05$). On assessment of the variables of autonomic dysfunction, higher fecal fat levels correlated significantly and positively with E:I ratio ($r = 0.58$, $p = 0.02$) whereas a significant negative correlation was observed with the 30:15 ratio ($r = -0.60$, $p = 0.02$).

4. Discussion

In this cross-sectional study, we report a high prevalence of EPI related fat malabsorption in patients with T2DM aged between 41 and 70 years using the standard 72-hours fecal fat assay method. Data from this study demonstrate significant correlation of EPI related fat malabsorption with variables of cardiac autonomic dysfunction in Asian Indians with Type 2 diabetes mellitus. Certain

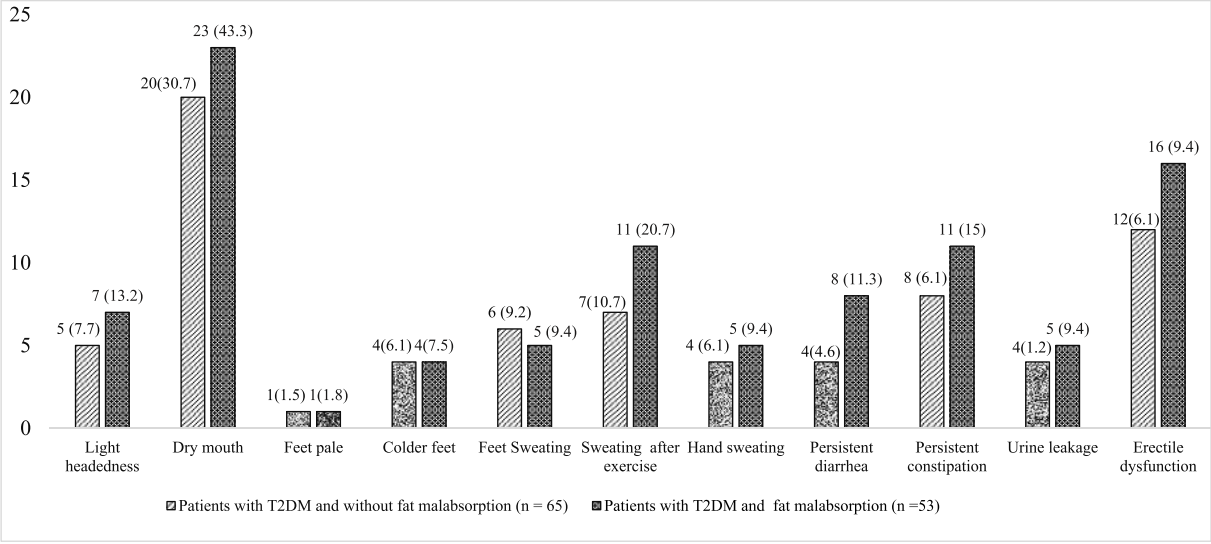

Fig. 3. Frequency of symptoms of Exocrine Pancreatic Insufficiency evaluated using the SAS questionnaire between groups.

Table 3
Assessment of Cardiac autonomic neuropathy between groups.

Cardiac Autonomic function parameters on CANS 504		Patients with T2DM and without fat malabsorption (n = 65)	Patients with T2DM and fat malabsorption (n = 53)	P value
Heart Rate	≤100	63 (97)	49 (92.4)	<0.05#
	≥101	2 (3.1)	4 (7.6)	
Heart rate in response to deep breathing. Expiration: Inspiration ratio (E: I)	Normal	26 [40]	11 (20.8)	< **0.01**#
	Borderline	20 (30.7)	18 (33.9)	
	Abnormal	19 (29.3)	24 (45.3)	
Heart rate in response to standing (30:15 ratio)	Normal	23 (35.4)	5 (9.4)	< **0.05**#
	Borderline	2 (3.1)	3 (5.7)	
	Abnormal	40 (61.5)	45 (84.9)	
Heart rate in response to Valsalva maneuver (Valsalva ratio)	Normal	42 (64.6)	35 (66)	< **0.05**#
	Borderline	4 (6.2)	2 (3.8)	
	Abnormal	0 (0)	3 (5.6)	
	Incomplete test	19 (29.2)	13 (24.6)	
Postural hypotension	Normal	46 (70.8)	36 (67.9)	0.34
	Borderline	9 (13.9)	5 (9.5)	
	Abnormal	10 (15.3)	12 (22.6)	
Sustained hand grip	Normal	21 (32.3)	9 (16.9)	0.58
	Borderline	19 (29.3)	17 [32]	
	Abnormal	25 (38.4)	**27 (50.9)**	
Frequency of autonomic dysfunction between groups				
Parasympathetic Nervous system (PNS) dysfunction		40/65 (61.5)	46/53 (86.7)	< **0.05***
Sympathetic nervous system (SNS) dysfunction		47/65 (72.3)	49/53 (92.4)	< **0.05***
PNS + SNS dysfunction		43/65 (66)	44/53 (83.1)	< **0.05***

Values are presented as n & % (in parentheses).
P value < 0.05: Statistically significant. # & *indicates *P* value of *t*-test and Chi square tests respectively.

studies have shown that nearly 60% of patients with type 1 and type 2 diabetes mellitus have EPI [3,32–34]. An earlier study from India had reported a prevalence rate of 29% EPI in patients with T2DM, using the FE-1 ELISA for fat malabsorption [32]. In addition, a meta-analysis of prospective studies had previously demonstrated an EPI prevalence rate of 26.2% in patients with T2DM, without adjusting for confounders [35].

EPI in patients with diabetes is usually subclinical in nature and can be attributed to a complex interplay of factors such as poor glycemic control, duration of diabetes, and aging-related neuronal death [36]. However, there are conflicting reports regarding the association of EPI with duration of diabetes in patients with DM [37]. In the current study, the mean duration of diabetes in the cohort was ten years and nearly fifty percent of patients with T2DM had type 2 diabetes for more than five years. The 72 hours mean fecal fat value in patients with T2DM correlated significantly with duration of diabetes. In contrast, a study in patients with diabetes from Germany reported the lack of significant association of EPI with duration of diabetes specifically in patients with mean fecal fat excretion above 10 g per day [38]. The conflicting results between our study and other study [29] could be due to inter-ethnic differences, method of assessment of EPI, age at diagnosis of diabetes mellitus, dietary factors or genetic variabilities. Notably, the previously mentioned study [29] had used FE-1 ELISA for the diagnosis of EPI, whereas the 72-hours fecal fat assay was used in the current study which can detect mild to severe EPI related fat malabsorption.

The association of EPI with micro and macrovascular complications of T2DM has been researched earlier. In patients with T2DM, an independent and significant association of exocrine insufficiency with BMI and history of vascular disease has been evidenced [36]. A study in patients with T2DM (mean age 47.5 ± 8.9 years) found a significant association of exocrine insufficiency with retinopathy and measures of glycaemia, whereas no significant association was observed with peripheral neuropathy [39]. On the contrary, in this study we demonstrate a significant association between EPI associated fat malabsorption, glycosylated hemoglobin and peripheral neuropathy. This is plausible, as chronic hyperglycemia, oxidative stress and inflammation impede the regenerative process of the enteric nervous system, leading to steatorrhea, and EPI in patients with diabetes [40,41].

In patients with T2DM, autonomic dysfunction is multifactorial and often an underdiagnosed complication, requiring multiple tests to assess both the sympathetic and parasympathetic functions, especially in patient with long duration of the disease [40]. It has been proposed earlier that autonomic neuropathy in patients with diabetes causes exocrine insufficiency [11]. Patients with T2DM and EPI may present with sympathetic predominance in the early stages of autonomic dysfunction. Furthermore, autonomic neuropathy and secondary gastro-paresis can impair entero-pancreatic reflexes, which may mediate the postprandial exocrine pancreatic response [42].

Autonomic neuropathy in patients with T2DM can be evaluated symptomatically using the SAS questionnaire, prior to objective clinical assessment. In the current study, we recorded a significantly higher mean SAS score ($P < 0.05$) and a total impact SAS score ($P < 0.01$) in patients with T2DM who frequently reported symptoms of autonomic dysfunction. A similar study reported significant association of the SAS score with measures of autonomic function and abnormal 30:15 ratio in patients with impaired glucose tolerance and early onset of diabetic neuropathy [30]. We confirmed the observations of the SAS questionnaire using the computerized autonomic neuropathy symptoms analyzer (CANS 504). An objective assessment of autonomic neuropathy revealed significantly higher prevalence of PNS dysfunction as demonstrated by higher frequency of 30:15 ratio (92.4%), abnormal heart rate response to standing (84.9%), and deep breathing (45.3%) in patients with T2DM and EPI associated fat malabsorption. In addition, both parasympathetic and sympathetic autonomic dysfunctions were noted to be higher in patients with T2DM and EPI related fat malabsorption. Further, the 72 hours mean fecal fat level correlated significantly with peripheral neuropathy, the coefficient of variation of RR intervals during deep breathing, expiration and inspiration ratio, the 30:15 ratio and glycemic status, irrespective of confounders.

Variables such as blood pressure response to postural changes while sitting, standing, the ratio of R waves at 30 and 15 seconds (30:15 ratio), the RR ratio, the Valsalva ratio and a sustained

handgrip measured for a specified time, have been demonstrated as confirmatory tests of autonomic neuropathy [43]. An earlier study had demonstrated that the prevalence of autonomic neuropathy was nearly 60% in Asian Indian patients with T2DM and peripheral neuropathy. Further, the risk factors for CAN in T2DM patients were prolonged QTc, higher age and disease duration of more than ten years [44].

Physiologically, EPI is caused due to an upsurge in levels of proinflammatory cytokines such as tumour necrosis factor-alpha, Beta interleukin-1, and interleukin-6, leading to pancreatic inflammation in patients with T2DM. This also leads to a loss of appetite in patients with chronic heart failure, suggesting a possible role of EPI in cardiac autonomic neuropathy [45], especially in patients with T2DM. In addition, impaired entero-pancreatic reflexes due to visceral neuropathy, atrophy of the pancreas, fibrosis and loss of acinar cells, eventually result in exocrine pancreatic insufficiency [5,46], eventually leading to fat mal-absorption in patients with diabetes/

In summary, we report a higher prevalence of EPI related fat malabsorption in a cross-sectional sample of patients with T2DM, by the 72-hours fecal fat estimation. The results demonstrated significant correlation of increased fecal fat with parameters of autonomic neuropathy, suggesting as a possible causative mechanism for subclinical EPI in patients with long duration of T2DM. However, these preliminary data need to confirmed in trials with more robust design.

4.1. Limitations of the study

We acknowledge the limitations as this study is cross-sectional in design and the was not validated with FE-1 ELISA in a subset of the sample. Moreover, as this study relies on a 72-hours whole volume of fecal sample, it cannot be applied in hospitals without laboratory and cold storage facilities. In addition, the pancreatic enzymes namely lipases, amylases and proteases need to be measured in patients with diabetes and chronic fat malabsorption for a confirmative diagnosis of exocrine insufficiency. Furthermore, a comparative arm of patients with T2DM and without autonomic neuropathy would have been ideal for comparative analyses of clinical data between groups. However, this is beyond the scope of the current study and therefore we plan to perform prospective studies in future to validate this relationship.

Nevertheless, the observations of the current study provide baseline data for a prospective study to evaluate the response to pancreatic enzyme supplementation in T2DM patients with EPI. This will aid in unravelling the clinical significance of EPI in relation to clinical outcomes, especially glycemic control.

Contribution of authors

RD conceptualized the study. SA, RR and RD performed the study and drafted the manuscript. GR analyzed and interpreted the data, FJ and MEK contributed to discussion, BKA supervised the biochemical assays. RD, FJ, SA and NT reviewed and edited the manuscript.

Conflict of interest and authorship conformation form

Please check the following as appropriate:

✓ All authors have participated in (a) conception and design, or analysis and interpretation of the data; (b) drafting the article or revising it critically for important intellectual content; and (c) approval of the final version.

✓ This manuscript has not been submitted to, nor is under review at, another journal or other publishing venue.

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Declaration of competing interest

The authors declare that they have no conflicts of interest pertaining to this study.

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