ORIGINAL DATA

Microangiopathy is Common in Submucosal Vessels of the Colon in Patients With Diabetes Mellitus

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■ Abstract

OBJECTIVES: The pathophysiology behind gastrointestinal dysmotility in diabetes mellitus is unknown. Both esophageal dysmotility and gastroparesis have been shown to be associated with retinopathy, suggesting that microangiopathy is important in the common etiology. The aim of the present study was to examine whether patients with diabetes exhibit microangiopathy in the colon, and if present, to correlate microangiopathy with the clinical picture. **METHODS**: Consecutive patients subjected to colon surgery were identified in the southernmost districts of Skåne between January 2011 and May 2013. Medical records were scrutinized, and patients with a history of diabetes were noted. Gender, age, type of diabetes, treatment, complications, and other concomitant diseases were registered. Histopathologic re-evaluation of surgical biopsies with morphometric analyses of submucosal vessels in the colon was performed. Morphometric examination and clinical data were compared with non-diabetic patients. **RESULTS**: Of 1135 identified patients during the time period studied, 95 patients with diabetes were recognized and included. Fifty-three non-diabetic, randomly chosen patients served as controls. The mean age was 71.8 ± 10.2 and 71.4 ± 9.5 years in diabetic and non-diabetic patients, respectively. Microangiopathy was found in 68.4% of diabetic patients and in 7.5% of non-diabetic patients ($p < 0.001$). The wall-to-lumen ratio was 0.31 (0.23-0.46) in patients with diabetes compared with 0.16 (0.12-0.21) in non-diabetic patients ($p < 0.001$). No clinical association with microangiopathy could be verified. **CONCLUSION**: Microangiopathy in the colon is more common in diabetic than in non-diabetic patients. The clinical significance of microangopathy has yet to be clarified.

Keywords: diabetes · colon · microangiopathy · submucosal vessel · interstitial cell · retinopathy · gastroparesis

1. Introduction

Gastrointestinal dysmotility is a common complication in diabetes mellitus. Studies have shown that up to 30% of patients with diabetes suffer from gastroparesis, which is characterized as prolonged gastric emptying without mechanical obstruction [1]. The pathophysiology is supposed to be multifactorial and includes vagal neuropathy, hyperglycemia, loss of enteric neurons, loss of neuronal nitric oxide expression, smooth muscle abnormalities, and disruption of the interstitial cell of Cajal (ICC) networks [1, 2]. These changes may be secondary to the initial damage, and causality has not yet been clarified. Other diabetic complications are characterized by micro- or macrovascular damages [3]. A vascular origin for the gastrointestinal dysmotility has been discussed, but not thoroughly examined. Associations between gastroparesis and retinopathy, cardiovascular disorders, and hypertension have been observed [4, 5], but no association has been found between gastrointestinal dysmotility and retinopathy [6]. Intima-media thickness (IMT) and ankle

brachial pressure index (ABI), which are risk indicators of future vascular event [7], correlate with the gastric emptying rate [8].

Recently, esophageal dysmotility has been observed in the majority of patients with diabetes [9, 10]. Esophageal dysmotility, but not gastroparesis, was associated with retinopathy, independently of other risk factors [10]. Furthermore, anorectal dysfunction in diabetes was found to be associated with microangiopathy [11].

The aim of the present study was therefore to examine whether patients with diabetes exhibit microangiopathy in the colon, and if present, to find out whether microangiopathy is correlated with the clinical picture.

2. Material and methods

This study was performed according to the Helsinki declaration and approved by the Ethics Review Board of Lund University. All patients gave their informed consent before entering the study.

2.1 Study design

Consecutive patients undergoing surgery of the colon at any of the Departments of Surgery in the southernmost districts of Skåne between January 2011 and May 2013 were identified. All medical records were scrutinized, and patients with a history of diabetes were noted. Gender, age, type of diabetes, treatment, diabetic complications, other concomitant diseases, and gastrointestinal symptoms were registered. Also, histopathologic re-evaluation of surgical biopsies with morphometric analyses of submucosal vessels in the colon was performed. The morphometric examination and clinical data were compared with biopsies and data from non-diabetic patients.

2.2 Subjects

In total, 1135 patients were identified during the time period studied. Of these, 105 suffered from diabetes, and 95 were finally examined when cases with ischemic, necrotic biopsies were excluded. Fifty-three randomly chosen patients without diabetes were used as controls. None of the patients had received radiation therapy or major gastrointestinal surgery prior to the actual surgery. One or two patients in each group had received chemotherapy prior to surgery.

Abbreviations:
ABI – ankle brachial pressure index
BRB – blood-retinal-barrier
ENS – enteric nervous system
IBD – inflammatory bowel disease
ICC – interstitial cell of Cajal
IMT – intima-media thickness
IQR – interquartile range
NS – not significant
SD – standard deviation

2.3 Methods

Tissue for light microscopy was fixed in 4% neutral formalin. Standard sections from the colon resection tissue were cut at 4 µm and stained with hematoxylin and eosin. All samples were taken from areas with normal macro- and microscopic appearance and from diverticulum-free normal parts of the colonic specimen. Morphometric analysis was made with the use of the light microscope Olympus BX53 with 10x magnification (NY Microscope Co., NY, USA). In each case, at minimum three arterioles with a circular cross-section were selected, and their outer and inner circumferences were traced manually, followed by calculation of parameters including wall thickness and wall-to-lumen ratio [12]. A wall-to-lumen ratio ≥ 25% is classified as microangiopathy (arteriolosclerosis) [13]. We classified the changes as mild, moderate, and severe when the degree of luminal compromise was 25-30%, 31-50%, and >50%, respectively.

2.4 Statistical methods

All variables were analyzed for normal distribution by the Kolmogorov-Smirnov test. As normality was rejected in all variables except age the Mann-Whitney U-test was used. Values are expressed as median (interquartile range (IQR)) or mean ± standard deviation (SD) when appropriate. Categorical variables were examined by Fisher's exact test. $P < 0.05$ was considered statistically significant.

3. Results

3.1 Patient characteristics

There was no difference in age or gender distribution between patients with diabetes and non-diabetic patients (**Table 1**). The type of diabetes and complications are shown in **Table 1**. Since many of the patients were referred from private

clinics and small hospitals to a tertiary center for surgery, information about diabetes complications and clinical data were often missing in the medical records at Skåne University Hospital (**Table 1**). Two of the patients had diabetes onset after surgical removal of the pancreas, and were not classified as type 1 or type 2 diabetes.

Of the 36 patients (38%) with known retinopathy, two had macula edema and two had proliferative retinopathy. The rest of the patients had background retinopathy. Macroangiopathy was present in 52 patients (55%); myocardial infarction and/or angina pectoris (n = 20), stroke (n = 12), atrial fibrillation (n = 10), heart failure (n = 9), and peripheral arterial disease (n = 6) were the most common complications. Data on microalbuminuria were not available, which led to the definition of nephropathy as elevated creatinine levels without any explanation for renal disease other than diabetes and/or presence of microalbuminuria. Apart from diabetes, the patients also suffered from several other diseases, including hypertension (n = 59, 62.1%), ischemic heart disease (n = 37, 38.9%), hyperlipidemia (n = 14, 14.7%), and stroke (n = 12, 12.6%).

Of the diabetes patients, 63 received colon surgery due to colon cancer, 38 of which were located in the right-sided colon and 25 in the left-sided colon. The biopsies not related to colon cancer included samples from patients operated on diverticulosis (n = 9), adenomas (n = 6), Crohn's disease (n = 5), ischemia/ileus (n = 4), ulcerative colitis (n = 3), and one each of carcinoidosis, lymphoma, cancer in the appendix, pancreatic cancer, and rectum amputation.

In non-diabetic patients, 17 of the cancers were located in the right-sided colon and 13 in the left-sided colon. Other surgical diagnoses included adenomas (n = 6), diverticulosis (n = 4), appendicitis (n = 3), Crohn's disease (n = 3), ischemia/ileus (n = 3), neuroendocrine tumor (n = 2), constipation (n = 1), and hysterectomy with a partial colon resection (n = 1). Apart from the abovementioned diseases, the controls also suffered from other diseases; the most prevalent of which were hypertension (n = 20, 37.7%), any other type of malignancy (n = 12, 22.6%), ischemic heart disease (n = 10,

Table 1. Patient characteristics

Characteristic	Diabetes patients (n = 95)	Non-diabetic patients (n = 53)	p
Age (yr)	71.0 ± 10.2	71.4 ± 9.5	NS
Male/female	55/40	28/25	NS
Diabetes type (1/2)	3/90	-	-
Duration (yr)*	9.5 (4.0-15.2)	-	-
Treatment (n, %)			
Dietary	15, 15.8	-	-
Peroral antidiabetics	40, 42.1	-	-
Insulin	24, 25.3	-	-
Both peroral and insulin	16, 16.8	-	-
Complication (Yes/no/unknown)			
Macroangiopathy	52/42/1	-	-
Autonomic neuropathy	4/11/80	-	-
Nephropathy	33/39/23	-	-
Peripheral neuropathy	8/12/75	-	-
Retinopathy	36/43/16	-	-

Legend: Values are mean ± SD. Differences were calculated with Mann-Whitney *U*-test or Fischer's exact test. P < 0.05 was considered statistically significant. * Missing value n = 13.

18.9%), cholecystectomy (n = 7, 13.2%), hyperlipidemia (n = 5, 9.4%), and hypothyroidism (n = 5, 9.4%). The prevalence of other concomitant diseases was equal in both groups (data not shown), except for hypertension, which was higher in diabetes (p = 0.006).

3.2 Morphometric examination and associations

More diabetic than non-diabetic patients had signs of microangiopathy in the submucosal vessels (**Figure 1**, **Table 2**). According to the microangiopathy, the wall was thicker (p < 0.001), the lumen was more narrowed (p = 0.003), and thus the wall-to-lumen ratio was smaller (p < 0.001) in patients with microangiopathy compared to patients without this sign. When classifying microangiopathy into mild, moderate, and severe, 21 of 64 patients with diabetes (33%) had a wall-to-lumen ratio of >50%, whereas in non-diabetic patients, three of the four patients with microangiopathy had a ratio between 26% and 30%, and one had a ratio of 40%. Inflammation around the vessels was seen in four patients with diabetes and in two non-diabetic patients. Thrombosis was seen in one patient with diabetes.

Table 2. Histopathological characteristics

Characteristic	Diabetes patients (n = 95)	Non-diabetic patients (n = 53)	p
Wall (μm)	40.0 (28.0-56.0)	20.0 (14.0-22.5)	<0.001
Lumen (μm)	120.0 (82.0-172.0)	110.0 (85.5-145.5)	NS
Wall-to-lumen ratio	0.31 (0.23-0.46)	0.16 (0.12-0.21)	<0.001
Microangiopathy (n, %)	65, 68.4	4, 7.5	<0.001
Cancer (n, %)	64, 67.4	30, 56.6	NS
Localization (n, %)			
Cecum	28, 29.5	12, 22.6	-
Ascendens	21, 22.1	16, 30.2	-
Transversum	5, 5.3	5, 9.4	-
Descendens	9, 9.5	4, 7.5	-
Sigmoideum	32, 33.7	16, 30.2	-

Legend: Values are median (interquartile range (IQR)). Differences were calculated with Mann-Whitney *U*-test or Fischer's exact test. P < 0.05 was considered statistically significant.

In the patients with diabetes, there was no difference in age between those with or without microangiopathy (p = 0.190), or in the duration of the disease (p = 0.402). There was no association between microangiopathy and any other diabetic complication (data not shown). The presence of microangiopathy was not associated with tumor localization, and through this with the site of examination (p = 0.788), cancer (p = 0.816), gender (p = 1.000), or diabetes type (p = 0.790), but there was a trend towards higher prevalence of microangiopathy in the group receiving peroral diabetes medication (p = 0.069). It was not possible to find any information about gastrointestinal symptoms and function from the medical records.

There was no association between the presence of hypertension and microangiopathy in patients with and without diabetes (p = 1.000 and p = 0.627, respectively). After exclusion of all patients with hypertension or inflammatory bowel disease (IBD), the prevalence of microangiopathy was still more common in diabetes patients than in non-diabetic patients (p < 0.001 in both calculations).

4. Discussion and conclusions

This study shows that 68.4% of the diabetes patients had microangiopathy in submucosal vessels of the colon, compared to 7.5% in non-diabetic patients. The microangiopathy was severe in 33% of the diabetes patients, whereas the changes were mild to moderate in non-diabetic patients. It was not possible to find an association between clinical findings and microangiopathy in this study.

Although there seems to be an association between different complications in diabetes, the exact mechanism and relationships between microangiopathy at different sites are not obvious [14, 15]. Retinopathy is seen in about 35% of diabetes patients. The most important factor to avoid retinopathy is the maintenance of glycemic control [16-18]. Hyperglycemia *per se* affects gastrointestinal motility and prolongs the gastric emptying rate. In turn, gastroparesis and dysmotility may cause poor metabolic control in the patients by nonregular emptying rates [1, 2]. The association between gastrointestinal dysmotility and retinopathy may thus be based on a poor glycemic control, with increased risk of developing retinopathy, rather than another common causality behind the two complications [4, 5, 10].

The duration of diabetes seems to be associated with the prevalence of complications in some studies [19]. The lack of association between colonic microangiopathy and diabetes duration in the present study is not in accordance with our previous results, where an association between esophageal complications (but not gastroparesis) and duration was found [10]. As the vast majority of the patients had type 2 diabetes, the exact onset of the disease, and thus the duration, may be uncertain. Furthermore, the information about duration of diabetes was missing for some of the patients. Possibly, diabetes duration may be more closely related to retinopathy and microalbuminuria [10, 19, 24] than to other complications [10, 20].

Gastroparesis is frequently accompanied by autonomic neuropathy [1]. In diabetes, neuropathic complications are caused by microvascular processes [18]. The enteric nervous system (ENS) is embedded in the bowel wall, and all pathophysiological factors described, i.e. loss of enteric neurons and ICCs [1, 2], may be secondary to microvascular processes in a similar way. As early as 1965, it was shown that the arterioles and capillaries of the submucosa showed the same pathologic changes and thickening in long-term diabetes as the blood vessels of other organs [21]. Other studies could not verify vascular complications in the

bowel mucosa [22], but mural thickening and luminal narrowing in blood vessels in duodenal biopsies have been described recently again [23]. However, this has not been discussed or further examined in relation to the etiology of gastrointestinal dysmotility.

Hypoxia has been supposed to cause the breakdown of the blood-retinal-barrier (BRB), edema, and a proliferative phase of retinopathy. Recent research suggests that ischemia in the tissues as well as biochemical and metabolic alterations within endothelial cells, pericytes, and smooth muscle cells are coupled with an impaired ability of the cells to perform self-repair, which may cause degenerative changes and/or apoptosis [24]. Retinal neuron and glia dysfunction are compromised before overt vessel changes are present, both in clinically situations and in experimentally induced diabetes in rodents [24]. Similarly, biochemical and metabolic processes may take place in enteric neurons and enteric glia cells, in addition to ischemia caused by angiopathy. Because of its inaccessibility, the bowel wall is much less studied than the retina. Apart from diabetes, malignant hypertension and transplantation with immune-modulating therapies may also cause the development of microangiopathy [25, 26]. However, these etiologies are rare. The conditions were not present in our study.

The main limitations of this study relate to the absence of an examination of gastrointestinal function and the lack of knowledge about related symptoms. The patients were relatively old or had actually deceased at the time of data analysis, they had malignancies that had spread such that they received chemotherapy, and they suffered from many concomitant diseases. Therefore, it was not possible to subject the majority of patients to further examinations with scintigraphy and manometry. Future studies ought to examine whether microangiopathy may be a causal risk factor for dysmotility. If this were verified, then it would be possible to treat gastrointestinal complications including drugs directed against hypoxemia and/or metabolic disturbances.

Another limitation is that the rectum was not examined. As rectal cancer is treated with radiation therapy prior to surgery, the tissue was un-
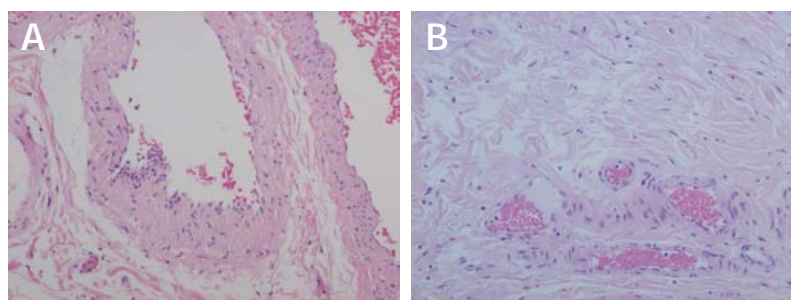
Figure 1. Microangiopathy in the arteriolar walls of the colonic submucosa. The figure shows sections of arteriolar walls, with increased thickening representing microangiopathy (**A**), and with normal thickening without microangiopathy (**B**). Sections visualized by light microscope Olympus BX53, magnification x 20, using hematoxylin and eosin staining.

suitable for histopathological evaluation of the vessels. The high amount of missing clinical values is a limitation of the study. Many patients were referred to a tertiary center for surgery, and medical records were not available for data about complications. The non-diabetic patients had a lower prevalence of hypertension than the diabetic patients, which is as expected since diabetes increases the risk for this complication. However, hypertension was not associated with the presence of microangiopathy.

Type 2 diabetes is associated with an increased incidence of colorectal cancer [27]. This may explain why cancer was more common, although not statistically significant, in patients with diabetes than in non-diabetic patients. In our cohort, the majority of cancers were right-sided tumors, although left-sided and rectal cancers had previously been more common. During the past decades, the incidence of right-sided tumors has increased [28]. Studies suggest that this is caused by an increased age at diagnosis, particularly in women, and an increased prevalence of obesity in the population [28, 29].

In conclusion, microangiopathy is very common in submucosal vessels of the colon in patients with diabetes. The exact mechanism behind the development and its effects on gastrointestinal function should be further investigated in future research.

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