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Comparison of Retinal Nerve Fibre Layer Thickness in Diabetic Patients with and without Diabetic Retinopathy Using OCT (Optical Coherence Tomography)

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Abstract: ***Purpose: This study intent to highlight the OCT characteristics of the RNFL in patients with diabetic retinopathy. To evaluate the association if any of RNFL thickness with diabetic retinopathy and to evaluate possibility of RNFL thickness changes as precursor to diabetic retinal changes. Methods and Methodology: This study was conducted at Department of Ophthalmology Chalmeda Ananda Rao Institute of Medical Sciences from Aug - 2019 to Jan - 2020.100 patients fulfilling inclusion and exclusion criteria were included in the study. All patients were subjected to detailed systemic and ophthalmic evaluation and Investigations like Blood Sugars Sr. Creatinine Sr. Cholesterol were done. Inclusion Criteria: Patients with diabetes mellitus over 40 years of age. Exclusion Criteria: Recent ocular surgery (1month) pseudo exfoliation pigment dispersion syndrome thyroid dysfunction long term steroid user's high myopia media opacities other causes for secondary glaucoma post glaucoma surgery. Results: The Average RNFL thickness in the study was 93.92 micrometres. Average inferior superior nasal RNFL thickness' is not significant across groups. The average temporal RNFL thickness in the study was 65.02 micrometres and it was significantly thicker (p0.01) across groups. Conclusion: An early increase intemporal RNFL thickness in some stages of diabetic retinopathy could be indicator of impending CSME.***

Keywords: Diabetic Retinopathy; Optical Coherent Tomography; Retinal Nerve Fibre Layer Thickness; Apoptosis; Type 2 Diabetes

1. Introduction

Diabetic retinopathy (DR) leads to direct damage to the nerve fibre layer, glial cells or neuronal metabolism which directly impact neurotransmission and may lead to apoptosis of retinal neurons, ischemia, reduced protein synthesis, depleted myoinositol, and high sorbitol levels have been demonstrated in patients with diabetes and may also result in nerve fibre loss in peripheral nerves [1].

Several studies have reported RNFL thinning or defects in people with diabetes that is not only caused by biochemical mechanisms that potentially cause neural cell degeneration but also by the laser photocoagulation that cause destruction of the retinal regions which leads to vascular abnormalities.

As this study discussing that Diabetic patients with mild NPDR showed decreased in thickness than normal subjects, also this decrease which is an early detection of RNFL thinning may help ophthalmologists to provide effective treatment of diabetic retinopathy and early prevention, thus reducing vision loss [2].

OCT performs, cross - sectional tomographic imaging in tissues, it is analogous to ultrasound B - mode imaging except that it uses light rather than sound. OCT performs imaging in biological tissues by directing an optical beam of infrared low coherence laser onto the tissue and measuring the reflected or backscattered intensity of light from microstructures within tissue as a function of depth [3].

Retinal nerve fibre layer (RNFL) measurements using optical coherence tomography (OCT) programs for nerve head indicate that the highest degree of variability can be attributed to interpatient differences. The recently developed OCT provides the ophthalmologist with the opportunity to customize scans and to tailor a single scan circle to examine RNFL thickness. Custom scans can be useful to help the ophthalmologist differentiate normal from early affected peripapillary RNFL.

2. Materials and Methodology

This study analysed 200 eyes of 100 adult patients whom their ages ranged from 40–90 years who attended the outpatients clinics at Chalmeda Institute of Medical Sciences from August 2020 – January 2021 and where The study was approved by the ethical committee of the scientific research, Subjects were given full explanations about the purpose of the study and its consequences and all patients were informed about the steps of the examinations and investigations

Patients were subjected to full ophthalmological examination including measurement of visual acuity aided and unaided, refraction, anterior segment examination using

slit lamp bio microscopy to detect any opacity or any other abnormality intraocular pressure measurement, and posterior segment examination using slit lamp with +90 lens.

Inclusion criteria:
Patients with diabetes mellitus
(Diabetes mellitus was diagnosed on the basis of the diabetes diagnostic criteria of the World Health Organization, and the patients were under medical treatment by an experienced physician/ endocrinologist)

Age > 40 years of age

Exclusion Criteria:
- Recent ocular surgery (1month)
- Patients <40years of age
- Patients with pseudo exfoliation
- Pigment dispersion syndrome,
- Thyroid dysfunction,
- Long term steroid users,
- High myopia,
- Media opacities like cataract,
- Other causes for secondary glaucoma,
- Post glaucoma surgery

OCT was done in 100 patients. For analysis purpose the data was taken as 200 eyes, which contains 50 eyes in the control group and 150 eyes in the Diabetic retinopathy group.

In this study patients were divided into 4 groups:
- Non - Diabetics (normal patients without Diabetes) - 25 patients (50 eyes)
- Diabetics without retinopathy (DNDR group) - 25 patients (50 eyes)
- Non proliferative diabetic retinopathy (NPDR group) - 25 patients (50 eyes)
- Proliferative diabetic retinopathy (PDR group) - 25 patients (50 eyes)

Patients with diabetes mellitus were divided into three groups on the basis of the international clinical diabetic retinopathy disease severity scale.

DNDR was defined as the absence of all features of diabetic retinopathy in diabetic eyes; NPDR was defined as the presence of microaneurysms, hard exudates, dot and blot haemorrhages, cotton wool spots, venous beading and intraretinal microvascularabnormalities (IRMA); and PDR was defined as the presence of neovascularization onoptic disc or elsewhere, vitreous or preretinal haemorrhage, and fibrovascular proliferative tissue.

All the patients are staged as mild NPDR by coloured fundus photography and FFA using Cannon fundus camera where mild NPDR showed presence of few dots of haemorrhages, microaneurysms and hard exudates without macular edema categorized as mild NPDR and the later phases of the test apart from the fovea excluding any macular edema.

Optical coherence tomography performed with Appasamy OCT using retinal nerve fibre layer thickness through 3D disc protocol measuring strategy after pupil dilation with 1% tropicamide and each eye had circular scans around the optic disc with a diameter of 3.4 mm. average of three qualified circular scans was used to calculate the overall mean total RNFL thickness from this 3d disc strategy.

3. Results

Table 1: Age Distribution

Demography

Age
The age of the patients in the study ranged from 40 to 90 years

Age (in years)	No: of Patients	Percentage
41 - 50	21	21%
51 - 60	38	38%
61 - 70	32	32%
71 - 80	8	8%
81 - 90	1	1%

Table 2: Sex Distribution
The study group had 65 males (65%) and 35 females (35%).

SEX	No: of Patients	Percentage
Male	65	65%
Female	35	35%

Retinal nerve fibre layer defect

RNFL defect present in 78 eyes out of 150 (52%) eyes of diabetic group

Table 3: Retinal nerve fibre layer defect

Group	
DNDR	21/50 (42%)
NPDR	31/50 (62%)
PDR	26/50 (52%)

Average retinal nerve fibre Layer thickness

Table 4: Average retinal nerve fibre Layer thickness across groups

Groups	Mean (+ SD)	F value	Significance
Non - Diabetic	93.26 (8.77)	1.54	0.205
DNDR	91.80 (9.80)		
NPDR	94.24 (19.65)		
PDR	96.36 (12.89)		

The average retinal nerve fibre layer thickness between groups were not statistically significant.

Inferior retinal nerve fibre layer thickness

Table 5: Inferior retinal nerve fibre layer thickness across groups

Groups	Mean (+ SD)	F value	Significance
Non - Diabetic	120.36 (13.38)	0.231	0.88
DNDR	118.78 (14.16)		
NPDR	121.50 (22.03)		
PDR	119.16 (21.26)		

The inferior retinal nerve fibre layer thickness between groups were not statistically significant

Superior retinal nerve fibre layer thickness

Table 6: Superior retinal nerve fibre layer thickness across groups

Groups	Mean (+ SD)	F value	Significance
Non - Diabetic	113.44 (13.79)	1.23	0.30
DNDR	117.48 (16.01)		
NPDR	120.02 (16.90)		
PDR	117.06 (21.56)		

The superior retinal nerve fibre layer thickness between groups were not statistically significant. Nasal retinal nerve fibre layer thickness

Table 7: Nasal retinal nerve fibre layer thickness across the groups

Groups	Mean (+ SD)	F value	Significance
Non - Diabetic	71.38 (9.18)	1.41	0.24
DNDR	69.46 (10.60)		
NPDR	72.66 (12.74)		
PDR	73.96 (12.66)		

The nasal retinal nerve fibre layer thickness between groups were not statistically
Significant

Temporal retinal layer thickness

Table 8: Temporal retinal nerve fibre layer thickness across the groups

Groups	Mean (+ SD)	F value	Significance
Non - Diabetic	61.48 (8.33)	11.45	<0.001
DNDR	62.08 (9.67)		
NPDR	61.78 (10.84)		
PDR	74.74 (21.33)		

Since there was a statistically significant difference across the groups for the temporal retinal nerve fibre layer thickness, between groups comparison of temporal retinal nerve fibre layer thickness was analysed.

Table 9: Between group comparison of temporal retinal nerve fibre layer Thickness

Groups	Comparison between the groups	Mean difference	p value
Non - Diabetic	DNDR	0.6	1.00
	NPDR	0.3	1.00
	PDR	13.26	<0.001
DNDR	NPDR	0.3	1.00
	PDR	12.6	<0.001
NPDR	PDR	12.96	<0.001

Temporal retinal nerve fibre layer thickness was significantly more ($p<0.01$) in PDR group when compared with the Non - Diabetic, PDR group versus DNDR group and in PDR group versus NPDR group. It is not significant between NON - DIABETIC group versus DNDR group, NON - DIABETIC group versus NPDR group, and DNDR group versus NPDR group.

Macular thickness

Table 10: Macular thickness across the groups

Groups	Mean (+ SD)	F value	Significance
Non - Diabetic	267.86 (17.39)	26.70	<0.001
DNDR	249.76 (28.94)		
NPDR	290.24 (42.69)		
PDR	305.96 (40.49)		

Since there was a statistically significant difference across the groups for themacular thickness, between groups comparison of macular thickness was analysed.

Table 11: Between group comparison of macular thickness

Groups	Comparison between the groups	Mean difference	p value
Non - Diabetic	DNDR	18.1	0.048
	NPDR	22.38	0.07
	PDR	38.10	<0.001
DNDR	NPDR	40.48	<0.001
	PDR	56.20	<0.001
NPDR	PDR	15.72	0.127

Macular thickness was significantly more ($p<0.01$) in PDR group when Compared to Non- Diabetic group, NPDR group to DNDR group and PDR group to DNDR group. It was not statistically significant in Non- Diabetic group versus DNDR group, Non – Diabetic group versus NPDR group and NPDR group versus PDR group.

Temporal retinal nerve fibre layer thickness

Table 12: Temporal Retinal Nerve Fibre layer thickness across the groups1

Groups	Mean (+ SD)	F value	Significance
Non - Diabetic	61.48 (8.33)	7.83	0.001
NCSME	63.32 (15.22)		
CSME	71.48 (16.16)		

Since there was a statistically significant difference across the groups for the temporal retinal nerve fibre layer thickness, between groups comparison of temporal retinal nerve fibre layer thickness was analyzed

Table 13: Between group1 comparison of temporal retinal nerve fibre layer Thickness

Groups	Comparison between the groups	Mean difference	p value
Non – Diabetic	NCSME	1.83	1.00
	CSME	9.99	0.001
NCSME	CSME	8.16	0.003

Temporal retinal nerve fibre layer thickness was significantly more in CSME group when compared to NON - DIABETIC group and CSME to NCSME groups. It was not significant in NON - DIABETIC versus NCSME group

Macular thickness

Table 14: Macular thickness across the groups1

Groups	Mean (+ SD)	F value	Significance
Non – Diabetic	267.86 (17.39)	53.14	<0.001
NCSME	262.61 (30.82)		
CSME	317.45 (43.63)		

Since there was a statistically significant difference across the groups for the macular thickness, between group comparison of macular thickness was analyzed.

Table 15: Between group1 comparison of macular thickness

Groups	Comparison between the groups	Mean difference	p value
Non – Diabetic	NCSME	5.26	1.00
	CSME	49.60	<0.001
NCSME	CSME	54.85	<0.001

Macular thickness was significantly increased (p<0.01) in CSME group when compared to Non - Diabetic group and CSME group to NCSME group. It was not significant in Non - Diabetic group versus NCSME group

4. Discussion

In the present study, most of the patients were in the age group of 40 - 90 years which accounted for 75% of the patients. Most of the patients were male patients accounting for 65% of the study group

Retinal Nerve fibre layer defect

Optic neuropathy is a well - known problem limiting visual acuity in diabetic patients. RNFL defect were detected by red free indirect ophthalmoscopy or red free slit lamp biomicroscopy and confirmed by OCT. In our study, RNFL defect (Table 3) was present in 78 eyes out of 150 (52%) eyes of diabetic group. RNFL defect was present in 21/50 (42%) of DNDR group, 31/50 (62%) of NPDR group and26/50 (52%) of PDR group. RNFL Defect was not found in any NON - DIABETIC group. This is in consistent with the study done by Chihara et al. [1] They photographed the retinal nerve fibre layer of the right eye of 137 patients with diabetes and 144 healthy control subjects. The level of diabetic retinopathy116 ranged from levels 1 (no microaneurysm) to 4 (eyes with localized intra - retinal micro vascular abnormalities or venous beading). Defects of the retinal nerve fibre layer were found in 6/30 (20%) eyes with level 1 retinopathy, 8/14 (57%) eyes with level 2 retinopathy, 24/47 (51%) eyes with level 3 retinopathy, and 36/46 (78%) eyes with level 4 retinopathy. These findings suggest that the retinal nerve fibre layer abnormalities are common inpatients with early diabetic retinopathy. They reported the risk factors for RNFL defect as a higher level of diabetic retinopathy, systemic hypertension, and advanced age, but visual acuity, disc size, axial length, and HbA1c level at the time of examination were reported to be not correlated with these defects. But inour study nerve fibre layer defect was present more in NPDR group than PDR group.

Retinal Nerve fibre Layer Thickness

In our study inferior RNFL thickness is thickest, and temporal is thinnest in NON – DIABETIC and DNDR groups. The superior and inferior areas were thicker because of the superior and inferior arcuate bundling of nerve fibers. In our study The Average retinal nerve fibre Layer thickness in the study is 93.92 micrometres and it is not significant across the groups (table 4). The Average inferior retinal nerve fibre layer thickness in the study is 119.95 micrometres and it is not significant across the groups (table 5). The Average superior retinal nerve fibre Layer thickness is in the study 117.0 micro meters and it is not significant across the groups (table 6). The Average nasal retinal nerve fibre Layer thickness is in the study is 71.87micrometres and it is not significant across the groups (table 7). The average, nasal and inferior RNFL thicknesses were decreased in DNDR group when compared to NON - DIABETIC group, but it is not statistically significant. The Average temporal retinal nerve fibre Layer thickness in the study is 65.02Micrometres and it is significant (p<0.01) across the groups (table8). The paired comparison of temporal retinal nerve fibre layer thickness in No diabetes mellitus (NON - DIABETIC) group and proliferative diabetic retinopathy (PDR) group was statistically significant (p<0.001) i. e. temporal retinal nerve fibre layer thickness is increased in PDR group compared to NON - DIABETIC group. The paired comparison of temporal retinal nerve fibre layer thickness in No diabetic retinopathy (DNDR) group and proliferative diabetic retinopathy (PDR) group was statistically significant (p<0.001) i.e., temporal retinal nerve fibre layer thickness is increased in PDR group compared to DNDR group. The paired comparison of temporal retinal nerve fibre layer thickness in non - proliferative diabetic retinopathy (NPDR) group and proliferative diabetic retinopathy (PDR) group was statistically significant (p<0.001) i.e. temporal retinal nerve fibre layer thickness is increased in PDR group compared to NPDR group (table 9). Temporal retinal nerve fibre layer thickness is increased in PDR group (74.74 +21.33). This is due to associated CSME. The paired comparison of temporal retinal nerve fibre layer thickness in No diabetes mellitus (NON - DIABETIC) group and DM with CSME group (CSME) group was statistically significant (p<0.001) i.e., temporal retinal nerve fibre layer thickness is increased in CSME group than in NON – DIABETIC group. The paired comparison of temporal retinal nerve fibre layer thickness in DM without CSME (NCSME) group and DM with CSME (CSME) group were statistically significant (p<0.001) i.e., temporal retinal nerve fibre layer thickness is increased in CSME group than in NCSME group. It was not significant in NON – DIABETIC versus NCSME group (table 13).

5. Macular thickness

The average macular thickness is 278.46 micrometres and it is statistically significant across the groups (table 18). The paired comparison of macular thickness in No diabetes mellitus (NON - DIABETIC) group and proliferative diabetic retinopathy (PDR) group was statistically significant (p<0.001) i.e. macular thickness is increased in PDR group compared to NON - DIABETIC group. The paired comparison of macular thickness in No diabetic retinopathy (DNDR) group and non - proliferative diabetic retinopathy (NPDR) group was statistically significant (p<0.001) i. e. macular thickness is increased in NPDR group compared to DNDR group. The paired comparison of macular thickness in No diabetic retinopathy (DNDR) group and proliferative diabetic retinopathy (PDR) group was statistically significant (p<0.001) i. e., macular thickness is increased in PDR group compared to DNDR group (table 19). The macular thickness is increased in both PDR group (305.96+ 40.49micrometres) and NPDR group (290.24 + 2.69 micrometres). The increase in thickness is due to

associated CSME. The paired comparison of macular thickness in No diabetes mellitus (NON - DIABETIC) group and DM with CSME group (CSME) group was statistically significant (p<0.001) i.e. macular thickness is increased in CSME groupthan in NON - DIABETIC group. The paired comparison of macular thickness in DM without CSME (NCSME) group and DM with CSME (CSME) group was statistically significant (p<0.001) i.e. macular thickness is increased in CSME group than in NCSME group. It was not significant in NON - DIABETIC versus NCSME group. It was not significant in NON - DIABETIC group versus NCSME group (table 26). The increase in macular thickness corresponds to increase in temporal retinal nerve fibre layer thickness in PDR group but not in NPDR group. This is again due to increased association of CSME with PDR group. This result is consistent with the study done by JeeTaek Kim et al [4], in which Retinal nerve fibre layer thickness and optic nerve head (ONH) in diabetic patients with normal tension were analysed using optical coherence tomography (OCT). There was an increase in the temporal average thickness of RNFL in the proliferative diabetic retinopathy group. Diabetic changes should be considered when diabetes patients are diagnosed with glaucoma or glaucoma progression. However, in subjects with very early glaucoma or in glaucoma suspects, the discriminating power of OCT might have been decreased because of thicker RNFL measurements affected by increased vascular permeability and changes in blood flow in diabetic retinopathy. Since the association of glaucoma and DM is quite common, this issue should be taken into account while assessing RNFL in diabetic glaucomatous patients. When a decrease in RNFL thickness is detected in a diabetic glaucoma patient one should consider the metabolic state of diabetes and the presence of retinopathy which may cause RNFL loss themselves before considering progression of glaucomatous damage in these patients. Sugimoto M et al [5]did a study to detect early diabetic damage in type 2 diabetes mellitus patients with no diabetic retinopathy (DNDR) using optical coherence tomography (OCT) and to evaluate OCT as a clinical test. They examined retinal and retinal nerve fibre layer (RNFL) thickness using OCT in thirty - two patients with DNDR. Two healthy normal populations were also enrolled for the retinal thickness (n = 48) and RNFL thickness (n = 34). OCT measurements were obtained in four areas (temporal, superior, nasal and inferior). The results of the study states that comparing the normal and DNDR eyes, retinal thickness (which involves all the layers of the retina) significantly increased (p = 0.03) and RNFL thickness significantly decreased (p = 0.02) in the superior areas. The area under the ROC curve was 0.65 for the superior retinal thickness and 0.63 for the superior RNFL thickness. There still remains a contrast with regard to the thickening of the retina that is seen in the macula compared to the thinning that is seen for the RNFL in the surrounding papilla. The macular region has a characteristic mechanism that differs from other retinal regions, i. e., a tendency to be oedematous. According to a histopathologic study, the Henle fibre layer consists of the Müller cell and axon in the macular region. Ischemia and other factors that might disrupt Müller cell function were implicated as a predisposing factor for the development of cystic macular edema. Thus, because of the abundance of Müller cells; the macular region is more fragile with regard to diabetic damage than the peripapillary region. The specific structure of the macula with no vasculature may be associated with the sensitivity to diabetic changes. They concluded that OCT might be used to detect much earlier signs and structural changes of DR. The results of our study are consistent with Hortensia Sanchez - Tocino et al [6] study. They did a study to quantitatively assess retinal thickness by optical coherence tomography (OCT) in normal subjects and patients with diabetes. This study was intended to determine which retinal thickness value measured with OCT best discriminates between diabetic eyes, with and without macular edema. OCT retinal thickness was measured by a manual technique in a total of 26healthy volunteers (44 control eyes) and 85 patients with diabetes (148 eyes) with the clinical diagnosis of no diabetic retinopathy (45 eyes), non - proliferative diabetic retinopathy without clinically significant macular edema (CSME; 54 eyes), and proliferative diabetic retinopathy without CSME (21 eyes), and 28 eyes with diabetic retinopathy with CSME. In this study there were statistically significant differences in foveal thickness between control eyes and all the other eye groups (P< 0.001). Eyes with NPDR or PDR had greater macular thickness in all regions than that in normal eyes. However, differences were not statistically significant in any of the areas. There were no significant differences in average thickness in any area between NPDR and PDR without CSME. Hee MR et al [7] have reported similar results, finding differences in central foveal thickness between normal eyes and eyes with diabetic retinopathy and no significant differences in average thickness between eyes with non - proliferative and proliferative diabetic retinopathy. However, they did not compare diabetic eyes with no diabetic retinopathy (DNDR) with normal eyes in healthy control subjects. Diabetic eyes with CSME had a statistically significant greater thickness in each of the areas compared with the other groups. In a multivariate logistic regression model, foveal thickness was a strong and independent predictor of CSME (odds ratio [OR], 1.037; 95% confidence interval [CI] 1.02–1.05). The area under the ROC curve of this predictor variable was 0.94 (P< 0.001). For a cut - off point of 180micro m, the sensitivity was 93%, and specificity was 75%. These results suggest that foveal thickening over 180 micro m measured by OCT may be useful for the
early detection of macular thickening and may be an indicator for a closer follow – up of the patient with diabetes.

6. Conclusion

The study titled “Retinal nerve fibre layer thickness in diabetic patients with and without diabetic retinopathy” was conducted at the Department of Ophthalmology, Chalmeda Institute of Medical Sciences. OCT was
done in 100 patients (200 eyes).

In this study patients were divided into 4 groups:

- Controls (normal patients without Diabetes) - 25 patients (50 eyes)
- Diabetics without retinopathy (DNDR group) - 25 patients (50 eyes)
- Non proliferative diabetic retinopathy (NPDR group) - 25 patients (50 eyes)

- Proliferative diabetic retinopathy (PDR group) - 25 patients (50 eyes)

The results of the study suggest that visual acuity of patients with diabetic retinopathy worsens with the stage of the retinopathy; CSME plays an important role in this worsening. Diabetic retinopathy was associated with a decrease in retinal nerve fibre layer thickness, though this is not statistically significant in our study, which is probably due to the number of patients with CSME in our study. There is also a possibility that the RNFL thickness and its changes with diabetes may be different in our south Indian population. Earlier studies show a statistically significant decrease in the RNFL thickness in patients with diabetic retinopathy.

However, in our study, temporal RNFL shows a significant increase in thickness, which worsens with the stage of diabetic retinopathy, this is due to the CSME which is associated with the retinopathy. The increase in macular thickness corresponds to increase in temporal retinal nerve fibre layer thickness in PDR group. This is again due to increased association of CSME with PDR group. The OCT takes into account the RNFL thickness at 3.4 mm from the disc margin and this is the zone where macular edema is usually very dense. As temporal RNFL shows a significant increase in thickness in some stages of diabetic retinopathy, an early increase in this thickness could be an indicator of impending CSME. Similarly while assessing a patient with glaucoma with diabetes, special care must be taken to evaluate the temporal RNFL thickness independently as this may be increased and should be excluded from analysis, as these values could in reality due to the diabetic changes in the retina and may skew the averages of the RNFL thickness while evaluating glaucoma [8]. Early detection of RNFL thickness changes caused by early diabetic retinopathy can leads to earlier treatments, although the existence of the first retinal changes does not have a therapeutic consequence [9], as we believe that OCT measurements may be one of the useful methods to elucidate the characterization of early structural changes of DR [10].

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