

Evaluation of nail fold capillaroscopy changes in patients with diabetic retinopathy and healthy controls, and its correlation with disease duration, HbA1c levels and severity of diabetic retinopathy: An observational study

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Abstract

Background: Diabetic retinopathy (DR) is an important microvascular complication of long-term type 2 diabetes mellitus (T2DM) leading to blindness if not properly diagnosed and managed. It can develop as early as 7 years before the diagnosis of diabetes. Nail fold capillaroscopy (NFC) is a non-invasive technique for observing capillary microvasculature and there are few studies which have explored the use of NFC in diabetes mellitus patients.

Objective: To study the nail fold capillaroscopic alterations in patients with T2DM having diabetic retinopathy and compare them to healthy controls. The secondary objective was to correlate the NFC findings with the duration of diabetes, haemoglobin A1c (HbA1c) levels and the severity of DR.

Materials and methods: This cross-sectional observational study enrolled 200 patients - 100 cases with T2DM having diabetic retinopathy (as per the American Diabetes Association criteria and Diabetic Retinopathy Disease Severity Scale) and 100 healthy age and sex-matched controls. All patients were subjected to NFC and ophthalmological assessment.

Results: NFC revealed that patients with DR showed significantly higher frequencies of tortuous, dilated, bushy, meandering, angulated capillaries, avascular areas and micro-haemorrhages as compared to healthy controls ($p < 0.05$). In proliferative DR (PDR), the frequency of tortuous, bushy capillaries, and avascular areas was statistically high and the capillary density was reduced as compared to non-proliferative DR. The DR patients with longer disease duration (>20) years had a significantly higher frequency of tortuous capillaries, avascular areas, meandering, angulated and dilated capillaries. The frequency of tortuosity, avascular areas, and bushy areas was significantly higher in patients with poor glycaemic control ($HbA1c > 11$).

Limitations: A larger sample size study with different demographic populations could have provided a broader picture of NFC changes in T2DM patients with DR.

Discussion: NFC may act as a surrogate marker of retinal involvement in patients with DM and should be performed at regular intervals.

Conclusion: NFC is a quick, simple, safe, and non-invasive method to assess the capillaroscopic alterations in diabetic patients which in turn can help in assessing the severity of DR.

Key words: Nail fold Capillaroscopy, Proliferative Diabetic Retinopathy, Non-proliferative Diabetic Retinopathy, Type 2 diabetes mellitus, HbA1c levels

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Introduction

Nail fold capillaroscopy (NFC) is a simple, non-invasive, painless *in vivo* technique to assess microvasculature. It is an established method to assess microcirculation in connective tissue diseases (CTDs) and helps in the early diagnosis and monitoring of Systemic sclerosis (SSc). The proximal nail fold, being an acral area, is affected early by disorders that affect microvasculature and has the advantage of easy accessibility for repeated examination.¹ Diabetic retinopathy (DR) is the most common microvascular complication of diabetes and remains a major cause of preventable blindness.² As compared to studies evaluating the retinal microvascular changes in diabetics, very little is known regarding the NFC features of these patients.³ The objectives of the present study were to assess the nail fold capillaries in diabetic retinopathy to evaluate the micro-vascular involvement, to identify if there is any correlation between nail fold capillaroscopic findings and the severity of DR and determine whether NFC changes have a relationship with the duration of diabetes and glycaemic index.

Materials and Methods

This cross-sectional observational study enrolled 200 patients that included 100 cases of DR and 100 healthy age and sex-matched controls and was conducted in the Department of Dermatology and Ophthalmology of a tertiary care institute over 1.5 years. Sample size calculation was done using convenience sampling. The Institutional Ethical Committee approved the study protocol (IEC No-3385/D-26/2020 Batch). Adults (>18 years) diagnosed with T2DM, based on the American Diabetes Association criteria (ADA)⁴ and DR as per Diabetic Retinopathy Disease Severity Scale⁵ and consenting to the study protocol, were included. Patients who did not give consent, those with type 1 diabetes mellitus, nail unit infections, trauma, patients with known CTD or on drugs affecting peripheral circulation, patients with aesthetic treatments in the last two weeks before the study, and pregnant and lactating females were excluded. Informed written consent was taken from all the patients, before their inclusion in the study [Figure 1].

A detailed NFC examination was done for all 10 fingernails, using a dermatoscope (AM 7515MZT Dino-Lite Edge Dermatoscope with 20x–220x magnification). The patients were made to sit for 15–20 min at an ambient temperature (around 20–22°C), with their hands at the level of the heart. Before the procedure nail plate and folds were cleaned with spirit and ultrasound gel was applied to the PNF to improve capillary visibility. NFC was performed, first at a low magnification for global visualisation of capillaries in the entire PNF and then at a higher magnification to assess individual capillary architectural change. Later the NFC changes were evaluated by the supervisor and recorded.

Qualitative morphological alterations in the capillary loops were visualised in all the fingers. The presence of dilated and giant capillaries (width of the capillary >2 times or >10 times

the normal respectively); tortuous (capillary limb curled but not crossing over), neo-angiogenesis/bushy capillaries (small, multiple buds originating from the distal loop); meandering (limbs crossed upon themselves several times); micro-haemorrhages, capillary dropouts; avascular areas (absence of two or more adjacent capillaries from the distal-most row); and bizarre capillaries (atypical morphology not conforming to the predefined morphologies) were recorded.^{6,7} Receding capillaries were seen as individual capillaries maintaining their position in the distal-most row but present slightly proximal to the distal-most loops. They represent that the capillary had receded backwards but had not dropped out yet. A special type of tortuous capillary reported was angulated capillary where one or both the arms of the distal capillary loop were seen to bend at an angle.³ The visibility of the subpapillary plexus was also recorded [Figures 2–8].

Results were tabulated and analysed objectively at the end of the study statistically. Categorical variables were presented in number and percentage, and continuous variables were presented as mean \pm SD. Quantitative variables were compared using an unpaired t-test between two groups. Qualitative variables were correlated using the Chi-square test. A 'p' value of <0.05 was considered statistically significant. The data were analysed using Statistical Package for Social Sciences (SPSS) version 22.0.

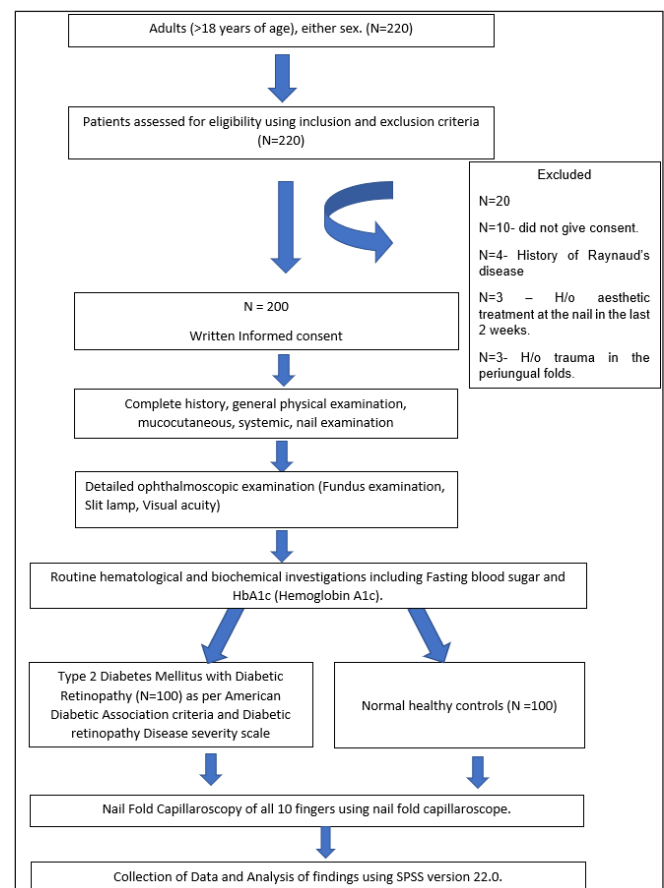


Figure 1: The protocol of the study conducted.

Results

A total of 100 patients with diabetic retinopathy and 100 healthy controls were evaluated and the baseline characteristics are summarised in Table 1.

The qualitative capillary changes in healthy controls and patients with DR are depicted in Table 1. The difference in the NFC changes between healthy controls and DR patients was statistically significant ($p = 0.001$).

Patients with DR were further subdivided into those with proliferative DR (PDR) and non-proliferative DR (NPDR) (mild, moderate and severe) as per Diabetic Retinopathy Disease Severity Scale⁵ and their nail fold changes were observed and analysed [Table 2]. Statistically significant morphological capillary alterations were observed in PDR as compared to NPDR [Figures 9 and 10].

The NFC parameters were compared in DR patients according to the duration of diabetes [Table 3]. The findings were found to be statistically significant ($p < 0.03$). Patients with DR were analysed based on their haemoglobin A1c (HbA1c) levels ($<7\%$, $7-9\%$, $9.1-11\%$, $>11\%$) reflecting their glycaemic control and the comparison is depicted in Table 4. Morphological capillary changes were more commonly seen in those with poor glycaemic control and were details are given in Table 4.

Discussion

NFC can be used to assess T2DM-related complications in the proximal nail fold capillaries.⁸ The most common qualitative capillary changes in healthy controls were tortuous, meandering, capillary dilation and micro-haemorrhages as observed in previous studies.^{3,9,10} In our study, the NFC

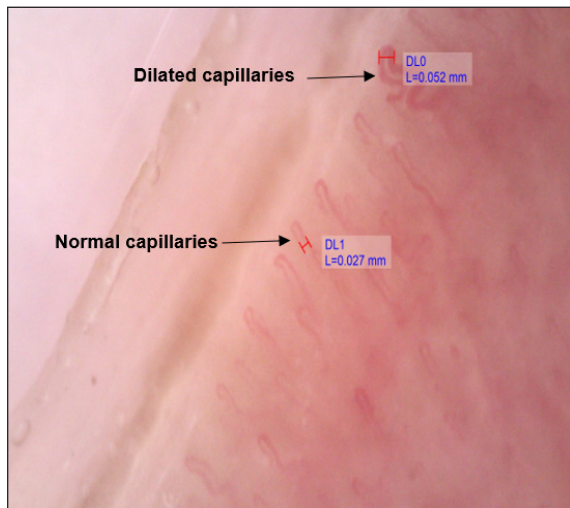


Figure 2: Nail fold capillaroscopy showing normal capillaries and dilated capillaries. (black arrow). [195.1 X] (DL0 – first dermoscopic calibration, DL1 – second dermoscopic calibration, H – represents the line used during the calibration.)

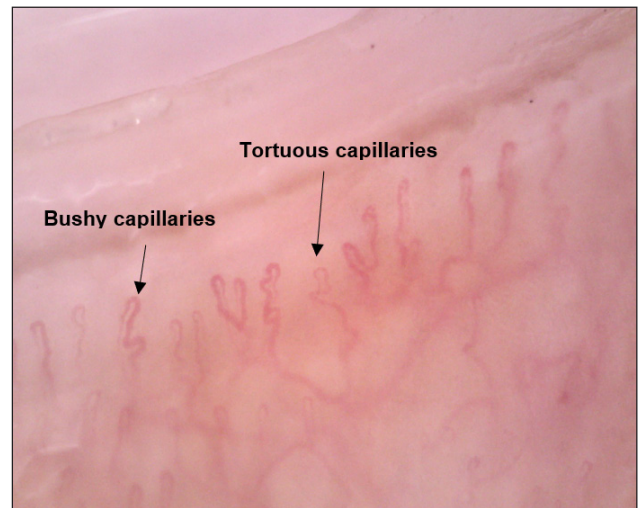


Figure 3: Nail fold capillaroscopy showing bushy capillaries and tortuous capillaries. (black arrow). [203.1x]

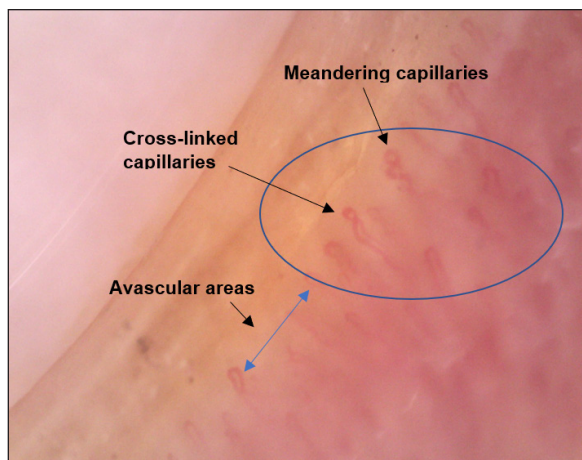


Figure 4: Nail fold capillaroscopy showing cross-linked (black arrow, blue circle), meandering capillaries (black arrow, blue circle) and avascular areas (blue arrow). [203.6 X]

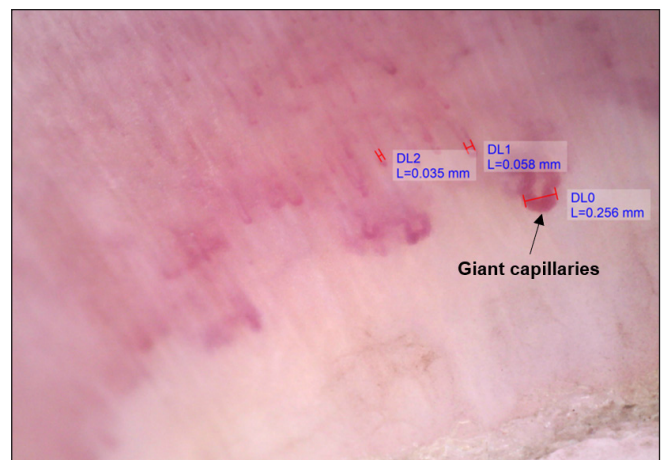


Figure 5: Nail fold capillaroscopy showing dilated and giant (black arrow) capillaries. [71.4x]. DL0: first dermoscopic calibration, DL1: second dermoscopic calibration, DL2: third dermoscopic calibration, H (red lines) signify the line used during calibration.

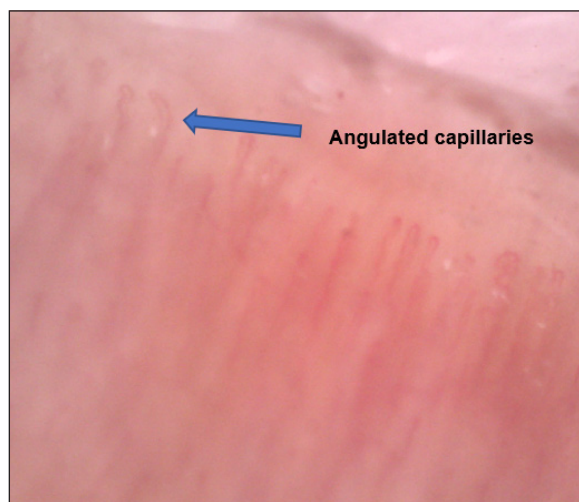


Figure 6: Nail fold capillaroscopy showing angulated capillaries (blue arrow). [149.8x]

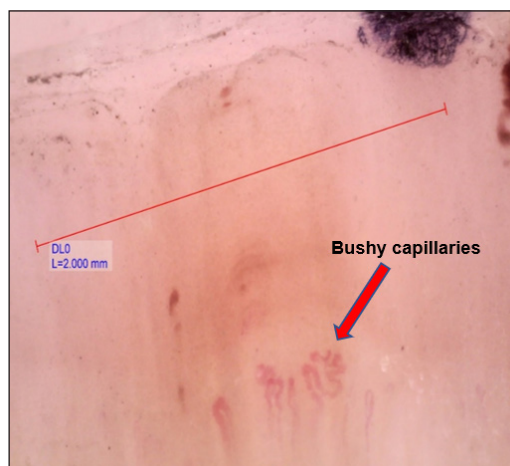


Figure 7: Nail fold capillaroscopy showing reduced capillary density and bushy capillaries (red arrow). [155.5x]. DL0: dermoscopic calibration used. H (red line) signify the line used during calibration.

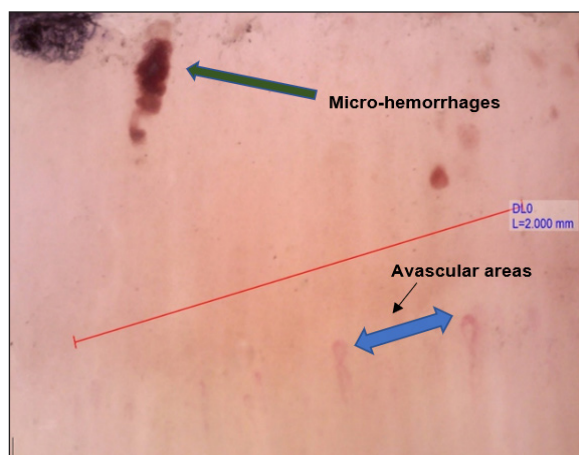


Figure 8: Nail fold capillaroscopy showing reduced capillary density, microhaemorrhages (green arrow) and avascular areas (blue arrow). [155.5x]. DL0: dermoscopic calibration used. H (red line) signify the line used during calibration.

changes were significantly higher ($p = 0.001$) in DR patients. Tortuosity, avascular areas, reduced capillary density, microhaemorrhages, and dilated, bushy, receding and angulated capillaries were common NFC findings in DR patients. The typical findings in diabetes may be due to the advanced glycation end-products (AGEs), which are known to be associated with macro- and microvascular complications.¹¹ One of the pathomechanisms is the apoptosis of retinal capillary pericytes and endothelial cells.¹² Contrarily, the well-known NFC features of CTD may be due to interaction between VEGF, antiangiogenic factors (angiostatin, endostatin, tumstatin, canstatin) and elevated levels of hypoxia-induced factor, which affects the capillaries and the surrounding connective tissue matrix.¹³ Thus, NFC features are regressive in advanced disease (capillary dropouts, avascular areas) and predominantly proliferative in early stages (capillary dilation, giant, bushy capillaries).

Maldonado *et al.* demonstrated that NFC changes in diabetic patients collaborated with ophthalmological findings in 18% of patients.¹⁴ A study done by Kaminska–Winciorek reported that the presence of mega capillaries, abnormal loops and altered capillary densities were seen more in DM of longer duration and high HbA1C.¹⁵ Various other studies have also recorded reduced capillary density, tortuosity, neoangiogenesis, haemorrhages, and avascular zones as the common findings in diabetics, which significantly correlated with the presence and degree of DR, duration and control of diabetes.^{16–20}

The findings of our study were consistent with those of Mohanty *et al.*, which reported tortuosity in 100% of patients with PDR and 81.8% with NPDR.²¹ In a study by Chang *et al.*, including 35 diabetic patients and 20 healthy controls, it was found that tortuosity was the most common finding in PDR (68%).²²

We studied the NFC features in DR patients according to the duration of diabetes. The findings were consistent with the study by Uyar *et al.* in which it was reported that diabetes years of patients having tortuosity, aneurysm, bizarre and micro-haemorrhage were significantly longer in patients with DR than without DR.⁸ Positive correlation of capillaroscopic findings and diabetes duration was also stated in Chang *et al.* study.²² The findings in our study are not consistent with the study done by Barchetta *et al.* in which NFC findings were independent of the duration of diabetes.¹⁶ Although these findings do not predict when the microvascular changes formed, there is a significant correlation between diabetes years and capillaroscopic findings in our study. It can be assumed that early detection of tortuosity, reduced capillary density, and dilated and microhaemorrhages may be a precursor of DR.

We studied the correlation of nail fold capillaries with the glycaemic index of the patient. The findings were consistent

Table 1: Baseline characteristics and comparative analysis of qualitative nail fold capillaroscopy features observed between cases (type 2 diabetics with retinopathy) and healthy controls

Baseline characteristics	Cases -Diabetic Retinopathy (n = 100), n (%)	Healthy controls (n = 100), n (%)	p-value
Mean age \pm SD (years)	58.32 \pm 9.00	56.43 \pm 7.62	0.926
Male: Female ratio	48:52	59:41	0.156
Mean disease duration \pm SD (years)	12.24 \pm 5.121	-	
PDR	14.52 \pm 4.229	-	
Severe NPDR	11.92 \pm 3.752	-	
Moderate NPDR	9.75 \pm 5.149	-	
Mild NPDR	6.20 \pm 4.658	-	
BMI	25.86 \pm 3.77	23.97 \pm 3.50	
Mean fasting blood sugar \pm SD (mg/dl)	177.01 \pm 48.40	94.91 \pm 3.75	
Mean HbA1c \pm SD (%)	8.80 \pm 1.60	5.43 \pm 0.21	
NFC parameters			
Presence of dilated capillaries	55 (55%)	6 (6%)	0.001*
Evidence of capillary dropouts	4 (4%)	7 (7%)	0.352
Presence of avascular areas	71 (71%)	3 (3%)	0.001*
Presence of micro-haemorrhages	27 (27%)	5 (5%)	0.001*
Bushy capillaries	42 (42%)	3 (3%)	0.001*
Meandering capillaries	33 (33%)	7 (7%)	0.001*
Tortuosity (>5% of capillaries)	82 (82%)	19 (19%)	0.001*
Reduced capillary density	61 (61%)	9 (9%)	0.001*
Subpapillary plexus visibility	10 (10%)	4 (4%)	0.096
Receding capillaries	19 (19%)	7 (7%)	0.012*
Angulated capillaries	31 (31%)	0	0.001*
Normal capillary distribution	9 (9%)	89 (89%)	0.001*

BMI: Body mass index, HbA1c: Haemoglobin A1c, SD: Standard deviation, NPDR: Non-proliferative diabetic retinopathy, PDR: Proliferative diabetic retinopathy
NFC: Nail fold capillaroscopy. *p-value<0.05 was taken as statistically significant.

Table 2: Comparative analysis of nail fold capillaroscopy features in diabetic retinopathy patients (According to the severity of Diabetic Retinopathy (DR)).

NFC parameters	NPDR (n = 50), n (%)				p value
	Mild (n = 5), n (%)	Moderate (n = 32), n (%)	Severe (n = 13), n (%)	PDR (n = 50), n (%)	
Presence of dilated capillaries	4 (80%)	16 (50%)	10 (76.92%)	25 (50%)	0.202
Evidence of capillary dropouts	0	0	0	4 (8%)	0.244
Presence of avascular areas	0	13 (40.63%)	11 (84.62%)	47 (94%)	0.001*
Presence of micro-haemorrhages	1 (20%)	1 (3.13%)	4 (30.77%)	21 (42%)	0.002*
Bushy capillaries	0	3 (9.38%)	4 (30.77%)	35 (70%)	0.001*
Meandering capillaries	0	0	2 (15.38%)	31 (62%)	0.001*
Tortuosity (>5% of capillaries)	2 (40%)	19 (59.38%)	11 (84.62%)	50 (100%)	0.001*
Reduced capillary density	0	3 (9.38%)	10 (76.92%)	48 (96%)	0.001*
Subpapillary plexus visibility	0	1 (3.13%)	1 (7.69%)	8 (16%)	0.230
Receding capillaries	0	0	0	19 (38%)	0.001*
Angulated capillaries	0	0	3 (23.08)	28 (56%)	0.001*

NFC: Nail fold capillaroscopy, NPDR: Non-proliferative diabetic retinopathy, PDR: Proliferative diabetic retinopathy. *p value <0.05 was taken as significant.

with the studies done by Hsu *et al.*,¹⁸ Uyar *et al.*⁸ and, Rajaei *et al.*²³ s which identified mega capillaries, increased tortuosity and neoangiogenesis as frequent changes in diabetic patients with longer duration and high HbA1c.

In a study done by El-Khalik *et al.*, there was a significantly longer disease duration and significantly higher HbA1c in

patients with DR than with non-DR.²⁴ Also, it was seen that the frequencies of tortuosity and precapillary oedema were increased significantly in patients with HbA1c \geq 7% compared to patients with HbA1c <7% and most of these patients were with diabetic retinopathy. This could be attributed to some studies showing HbA1c as a predictor of diabetic retinopathy.

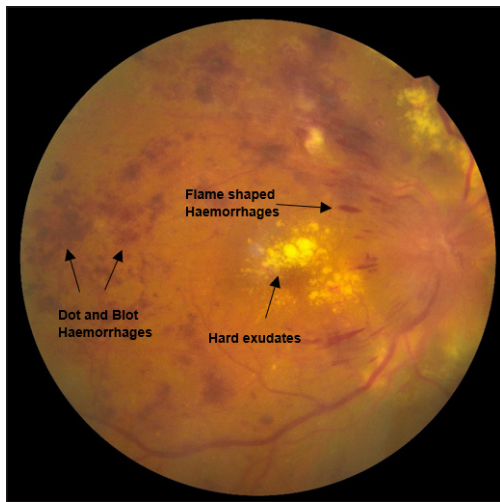


Figure 9: Fundus examination showing findings suggestive of proliferative diabetic retinopathy with clinically significant macular oedema (CSME). Black arrows suggesting dot and blot haemorrhages, flame shaped haemorrhages and hard exudates.

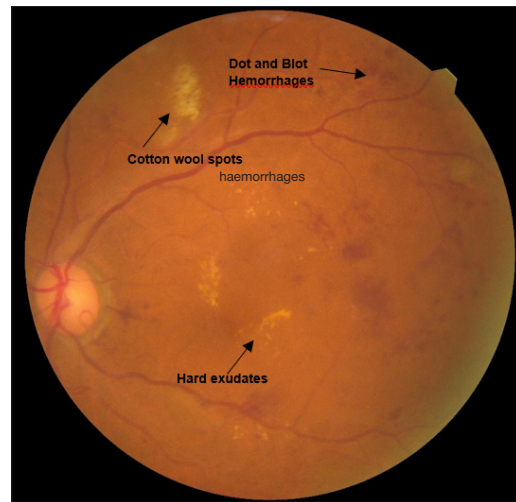


Figure 10: Fundus examination showing findings (black arrows suggesting dot and blot haemorrhages, cotton wool spots and hard exudates) suggestive of moderate nonproliferative diabetic retinopathy.

Table 3: Comparative analysis of nail fold capillaroscopy features in diabetic retinopathy patients (According to the duration of diabetes mellitus)

NFC Parameters	Duration of diabetes (in years)					P-value
	≤5 (n = 9), n (%)	6–10 (n = 27), n (%)	11–15 (n = 47), n (%)	16–20 (n = 13), n (%)	>20 (n = 4), n (%)	
Presence of dilated capillaries	6 (66.67%)	14 (51.85%)	24 (51.06%)	8 (61.54%)	3 (75%)	0.778
Evidence of capillary dropouts	0	0	3 (6.38%)	1 (7.69%)	0	0.588
Presence of avascular areas	1 (11.11%)	16 (59.26%)	38 (80.85%)	12 (92.31%)	4 (100%)	0.001*
Presence of micro-haemorrhages	0	5 (18.52%)	17 (36.17%)	3 (23.08%)	2 (50%)	0.112
Bushy capillaries	0	6 (22.22%)	27 (57.45)	7 (53.85%)	2 (50%)	0.003*
Meandering capillaries	0	4 (14.81%)	18 (38.30%)	8 (61.54%)	3 (75%)	0.03*
Tortuosity (>5% of capillaries)	3 (33.33%)	20 (74.07)	42 (89.36%)	13 (100%)	4 (100%)	0.001*
Reduced capillary density	0	10 (37.04%)	36 (76.60%)	12 (92.31%)	3 (75%)	0.001*
Subpapillary plexus visibility	0	0	8 (17.02%)	1 (7.69%)	1 (25%)	0.105
Receding capillaries	0	3 (11.11%)	12 (25.53%)	3 (23.08%)	1 (25%)	0.315
Angulated capillaries	0	4 (14.81%)	17 (36.17%)	7 (53.85%)	3 (75%)	0.005*

NFC: Nail fold capillaroscopy, *p value<0.05 was taken as significant.

Table 4: Comparative analysis of nail fold capillaroscopy features in diabetic retinopathy patients -according to the glycaemic index (HbA1c levels)

NFC parameters	HbA1c levels				P-value
	<7.0 (n = 15), n (%)	7.0–9.0 (n = 42), n (%)	9.1–11.0 (n = 36), n (%)	>11.0 (n = 7), n (%)	
Presence of dilated capillaries	6 (40%)	23 (54.76%)	24 (66.67%)	2 (28.57%)	0.150
Evidence of Capillary drop outs	0	0	3 (8.33%)	1 (14.29%)	0.109
Presence of avascular areas	8 (53.33%)	24 (57.14%)	32 (88.89%)	7 (100%)	0.002*
Presence of microhaemorrhagesC	2 (13.33%)	11 (26.19%)	12 (33.33%)	2 (28.57%)	0.537
Bushy capillaries	3 (20%)	12 (28.57%)	22 (61.11%)	5 (71.43%)	0.003*
Meandering capillaries	3 (20%)	5 (11.90%)	21 (58.33%)	4 (57.14%)	0.001*
Tortuosity (>5% of capillaries)	10 (66.67%)	30 (71.43%)	35 (97.22%)	7 (100%)	0.005*
Reduced capillary density	4 (26.67%)	17 (40.48%)	33 (91.67%)	7 (100%)	0.001*
Subpapillary plexus visibility	1 (6.67%)	4 (9.52%)	4 (11.11%)	1 (14.29%)	0.943
Receding capillaries	1 (6.67%)	4 (9.52%)	12 (33.33%)	2 (28.57%)	0.027*
Angulated capillaries	1 (6.67%)	8 (19.05%)	17 (47.22%)	5 (71.43%)	0.001*

NFC: Nail fold capillaroscopy, HbA1c: haemoglobin A1c, *P < 0.05 was taken as significant.

A study revealed that when HbA1c was $\geq 6.8\%$, the odds ratio for diabetic retinopathy increased significantly.²⁵

Limitations

A larger sample size study with different demographic populations could have provided a broader picture of NFC changes in type 2 DM.

Conclusion

Changes in NFC in diabetics had a significant association with the severity of DR, disease duration, and glycaemic control. These changes perhaps run parallel to the changes in the retina. Hence, nail fold capillaroscopy can be used as a valuable non-invasive tool not only for the diagnosis of microvascular complications but also for the prognosis of the disease and identifying patients at high risk of DR, thereby helping in early diagnosis and improving the quality of life of patients.

Ethical approval

The ethical approval number is 3385/D-26/2020 batch from Government Medical college, Amritsar, Punjab.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

Use of artificial intelligence (AI)-assisted technology for manuscript preparation

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript, and no images were manipulated using AI.

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