Histopathological Evidence of Thyroid Dermopathy and its Correlation with Thyroid-Associated Orbitopathy in Patients with Graves' Disease having Normally Appearing Pretibial Skin: A Case-Control Study

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Abstract

Objective: Thyroid dermopathy (TD), reportedly encountered in less than 5% of patients with Graves' disease (GD), is supposed to coexist only with thyroid-associated orbitopathy (TAO). However, clinically inapparent TD, detected non-invasively by thermal imaging or ultrasonography, seems to be present in a larger proportion of GD. Histopathological examination (HPE), though considered as gold standard for detecting TD, has not been performed widely to identify subclinical TD in GD. **Materials and Methods:** In this single-centre, cross-sectional, case-control study, 50 patients with GD (cases) and normal appearing pretibial skin were compared with 45 age- and sex-matched individuals (39 healthy volunteers, 3 with toxic multinodular goitre and 3 with solitary toxic nodule) (control). All patients were evaluated clinically for presence of TAO. Punch biopsy specimens were obtained from the pretibial skin in all 95 participants. Tissue sections were examined under light microscopy for mucin deposition, splitting of collagen fibrils and perivascular lymphocytic infiltration. **Results:** Sixty per cent of patients with GD demonstrated at least one of the above three histological features, while 52% had any combination of two features and 46% harboured all the three features. Mucin deposition, splitting of collagen fibrils and lymphocytic infiltration were found overall in 52%, 54% and 52% of GD, respectively; 4.4–11.1% of controls also had some evidence of TD on HPE. Subclinical TD was not related to age, duration of disease and TAO in our study. **Conclusions:** TD, particularly in its subclinical form, Seems to be widely prevalent in GD (46–60%) and exists even in absence of TAO. HPE, though more sensitive than the various non-invasive tests, is not specific (ranges from 89% to 95%) for TD. However, HPE can accurately diagnose TD in appropriate clinical background.

Keywords: Graves' disease, mucin deposition, perivascular lymphocytic infiltration, splitting of collagen, thyroid dermopathy

INTRODUCTION

Graves' disease (GD) is not only the commonest aetiology of hyperthyroidism but also the leading cause of thyrotoxicosis, accounting for 60–80% of such cases.^[1] Thyroid dysfunction in GD is often accompanied by infiltrative orbitopathy, also known as thyroid-associated orbitopathy (TAO), and occasionally by infiltrative dermopathy. The constellation of the thyroid, eye and skin signs is termed as the 'Graves' triad'. The prevalence of TAO in GD varies widely among studies, ranging from 25% in recent onset disease to as high as 100% when evaluated with orbital imaging.^[2] Many patients of GD,

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thus, have subclinical TAO that becomes apparent only after imaging.

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Thyroid dermopathy (TD) is usually obvious on clinical examination and is commonly located over the anterior and lateral aspects of lower leg, hence termed 'pretibial myxedema' (PTM); however, sites exposed to frequent trauma are also involved. PTM has four distinct clinical forms: diffuse non-pitting oedema (43%), raised plaque lesions (27%), sharply circumscribed tubular or nodular lesions (18%) and the rare elephantiasic form (35%).^[3] Lesions typically have 'peau d'orange' appearance and are deep pink or purple in colour. However, in dark-skinned individuals from the Indian subcontinent, such lesions are usually skin-coloured with areas of hyperpigmentation, hence might be missed during casual examination in inexperienced eyes. Clinically evident TD is thought to be encountered in less than 5% of patients with GD and seen almost always in presence of moderate-to-severe TAO.^[1,4] However, the true prevalence of TD seems to remain underestimated as clinically inapparent TD does exist. Such 'subclinical TD' was detected in a relatively larger proportion of patients by measuring pretibial skin thickness with ultrasonography (USG) (33%) or by measuring focal temperatures over the pretibial area using digital infrared thermal imaging (65%) or by identifying characteristic histopathological changes in punch biopsy specimen taken from the extensor surface of the forearm (55%).^[5-7] Histopathological examination (HPE) of a biopsied specimen obtained from the involved skin in an appropriate clinical background represents the gold standard for the diagnosis of TD. There are very few studies however, which looked at the prevalence of subclinical TD in GD. We tried to estimate such prevalence of subclinical TD on skin biopsy specimens in our cohort of patients with GD with or without clinically apparent TAO.

Aims and objectives

The study was undertaken to estimate the prevalence of subclinical TD in patients with GD. We also tried to determine any possible association between subclinical TD and clinically evident TAO in patients with GD.

MATERIALS AND METHODS

This cross-sectional, case-control, single-centre study was undertaken in the Department of General Medicine, Midnapore Medical College and Hospital, Midnapore, West Bengal, India. All patients with GD attending the out-patient department or admitted in the in-patient department from February 2019 were thoroughly evaluated clinically, and those without clinical dermopathy or any dermatological disease involving pretibial skin were enrolled in the study. Dermopathy was considered absent when two investigators examined the pretibial area separately and opined as normal. They were unaware about each other's findings.

Calculation of sample size

Prevalence of subclinical TD in an earlier cohort with GD was found to be 36%.^[5] To the best of our knowledge, none of the earlier studies looked into the presence of TD in individuals without GD. Minimum number of individuals (*N*) to be

enrolled in each group (case: control = 1:1) for this study was calculated by the following equation and was found to be 15.

$$N = [(p_0q_0 + p_1q_1)X(z_{1-\alpha/2} + z_{1-\beta})^2]/(p_1 - p_0)^2$$

Where p_0 is the prevalence of TD in those without GD (taken as 0); p_1 is the prevalence of subclinical TD in patients with GD (taken as 0.36); q_0 : $(1 - p_0)$; q_1 : $(1 - p_1)$; $z_{1-\alpha/2}$ is the 1.96 for two-sided 95% confidence level; and $z_{1-\beta}$ is the 0.95 for power of 95%.

Fifty patients with GD irrespective of thionamide (carbimazole) exposure were grouped as 'case'. The 'control' group included 45 individuals either with toxic multinodular goitre (TMNG) or solitary toxic nodule (STN) or healthy volunteers without any thyroid disease. Written informed consent was taken from all cases and controls. The study was approved by the institutional ethics committee (Memo No. MMC/IEC-2019/193 dated 28/01/2019).

GD was diagnosed based on suggestive symptoms of thyrotoxicosis for at least 3 months along with suppressed thyroid-stimulating hormone (TSH) (<0.27 µIU/ml) plus one or more of the following: diffuse enlargement of the thyroid gland with/without thyroid bruit, clinical features suggestive of TAO and hypoechoic thyroid with absence of nodule (more than 1 cm in largest diameter) with/without increased vascularity on USG. Diffuse and increased uptake of tracer in 99mTc-pertechnetate uptake and scan confirmed GD in equivocal cases with suppressed TSH. TSH receptor antibody (TRAb) was measured in a selected few. Diagnoses of TMNG and STN were based on scintigraphy. We did not perform orbital imaging, and the diagnosis of TAO was based only on clinical examination. We did not have access to Hertel exophthalmometer, hence could not measure proptosis. Presence of lateral eyelid flare or lower scleral show or restricted movement of one or more extraocular muscles (EOMs) (not due to cranial nerve palsy) indicated underlying TAO.^[8] Lateral flare has been suggested as a pathognomonic feature of TAO.^[2,9] Thyroid function tests (TFTs) were performed by electrochemiluminescence immunoassay in cobas e-411analyser (Roche Diagnostics, Basel, Switzerland). USG was done by LOGIQ P9 XDclear machine (GE Healthcare, USA) using 10 MHz probe.

TD was diagnosed by HPE of punch biopsy specimens taken from pretibial skin. Specimens obtained from sterilized skin biopsy punch (5 mm in diameter) were fixed in diluted formaldehyde solution, embedded in paraffin and cut into 2 μ m sections. The sections were then stained with hematoxylin and eosin stain. All slides were examined on a light microscope by the same pathologist, who was blinded about the clinical diagnosis. We focused on the following histological features: mucin deposition in dermis, splitting of dermal collagen fibrils and perivascular lymphocytic infiltration within dermis. Mucin deposition was subsequently confirmed by periodic acid–Schiff stain. A diagnosis of possible TD was made in presence of at least one feature, probable TD was considered if any combination of two features were present, while presence of all three features constituted definite TD.

Data analysis

For statistical analysis, data were entered in Microsoft Excel spreadsheet, Version 2019, and then analysed by SPSS, Version 20.0. Data were expressed as mean and standard deviation (SD) for normally distributed continuous variables and frequency and percentage for categorical variables. Student's t-test and Chi-square test were applied to compare mean and proportions (in percentage), respectively, to see the statistical significance. Point-biserial correlation coefficient (r)was used to correlate age of the study population (both cases and controls) and duration of GD (cases) with each of the three histological features. Value of r close to +1 or -1 indicated strong positive and negative association, respectively, while value of 0 suggested no association between variables. A P value < 0.05 was considered statistically significant. We also looked into the diagnostic accuracy of these three features on HPE for TD; hence, we calculated the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (PLR), negative likelihood ratio (NLR), and diagnostic odd ratio (DOR) of the three histological features, both in isolation and in different combinations. The following equations were used:

Sensitivity: [True positive (TP)/{TP + false negative (FN)}] \times 100

Specificity: [True negative (TN)/{false positive (FP) + TN}] $\times 100$

PPV: $\{TP/(TP + FP)\} \times 100$

NPV: $\{TN/(TN + FN)\} \times 100$

PLR: Sensitivity/(100 - specificity)

NLR: (100 - Sensitivity)/specificity

DOR: (TP/FN)/(FP/TN).

RESULTS

The mean age of the study population (n = 95) was 36.96 ± 11.07 years. Majority of them were female (n = 73; 76.8%) with the female: male ratio of 3.3:1. The 'control' population (n = 45) included healthy volunteers (n = 39); 86.7%), patients with TMNG (n = 3; 6.7%) and STN (n = 3; 6.7%). The demographic parameters, clinical findings and TFT of the study population have been summarized in Table 1. None of the patients with GD had digital clubbing. Mucin deposition, splitting of collagen and perivascular lymphocytic infiltration were found in 52%, 54% and 52% of patients with GD, respectively. Mucin deposition and splitting of collagen was the dominant combination in GD, seen in 52% of patients. One histological feature (possible TD) was seen in 60% (n = 30) and any combination of two features (probable TD) was seen in 52% (n = 26) of patients with GD. All the three features (definite TD) [Figure 1] were present in 46% of GD (n = 23), while



Figure 1: Light microscopy $(40 \times)$ showing mucin deposition (*), perivascular lymphocytic infiltration (solid circle) and splitting of collagen fibrils (dashed circle) in a patient with GD (haematoxylin and eosin stain) (a). Large mucin deposits (black arrow), stained as bright red, among collagen fibrils on periodic acid-Schiff stain $(40 \times)$ (b)

Table 1: Baseline characteristics of the study population (n=95)

Variables	Cases (<i>n</i> =50)	Controls (n=45)	Р
Age (years)	35.6±10.9	38.4±11.1	0.108
Male: Female	12:38	10:35	0.84
Duration (months)	14.8 ± 15.3		
Thyroid bruit	3 (6%)	0 (0%)	0.09
TAO	22 (44%)	0 (0%)	< 0.000001
Total T3 (nmol/l)	5.69 ± 3.49	2.36±1.03	< 0.000001
Free T4 (pmol/l)	49.43±30.76	18.02 ± 6.69	< 0.000001
TSH (µIU/ml)	0.08 ± 0.25	2.83±1.84	< 0.000001

Reference ranges: Total T3: 1.23-3.07 nmol/l, FT4: 12-22 pmol/l, TSH: 0.27-4.2 µIU/ml. T3: Triiodothyronine; T4: thyroxine; TAO: thyroid-associated orbitopathy; TSH: thyroid-stimulating hormone

40% (*n* = 20) harboured none of these features. Interestingly, 11.1% of control population also demonstrated possible TD, 6.7% had probable TD and 4.4% had HPE consistent with definite TD. In both the case and control groups, splitting of collagen was more frequent than the other two features. The prevalence of all the histological features, either in isolation or in combination, was significantly higher in GD compared to the control population [Table 2]. A subgroup analysis, however, did not establish any significant difference in histological findings between GD and those with toxic nodular thyroid disease (TMNG/STN). Splitting of collagen was the dominant histopathological feature seen in 90% of those with TD [Table 3]. Twenty-two patients (44%) of GD had TAO and histological findings were not different between those with and without TAO [Table 4]. Possible TD, probable TD and definite TD were seen in 68.2%, 59.1% and 54.5% of those with TAO, respectively. There was no correlation between age of participants and histological features of TD either in cases or controls [Table 5]. Similarly, duration of GD also had no effect on prevalence of TD [Table 6]. Half of the patients with GD was newly diagnosed and the remaining half was on carbimazole during recruitment; no difference in histological findings was found between these two groups [Table 7]. Duration of carbimazole therapy [Table 8] or severity of GD,

Table 2: Histopathological findings in the s	tudy popu	ılation (<i>n</i> =9	5)								
Variables	Cases	; (<i>n</i> =50)	Contro	ls (<i>n</i> =45)	ď	Control pop toxic nodula	ulation without r goitre (<i>n</i> =39)	٩	Control po TMNG/	pulation with STA (<i>n</i> =6)	ط
	Number	Percentage	Number	Percentage		Number	Percentage		Number	Percentage	
Mucin deposition (only)	0	0	0	0	NA	0	0	NA	0	0	NA
Mucin deposition (overall)	26	52	с	6.7	<0.00001	1	2.6	<0.00001	2	33.3	0.39
Splitting of collagen (only)	1	2	1	2.2	0.94	0	0	0.37	1	16.7	0.07
Splitting of collagen (overall)	27	54	4	8.9	<0.00001	1	2.6	<0.00001	с	50	0.85
Lymphocytic infiltrate (only)	3	9	1	2.2	0.36	1	2.6	0.44	1	16.7	0.34
Lymphocytic infiltrate (overall)	26	52	С	6.7	<0.00001	2	5.1	<0.00001	1	16.7	0.10
Mucin deposition + splitting of collagen (only)	3	9	1	2.2	0.36	0	0	0.12	1	16.7	0.34
Mucin deposition + splitting of collagen (overall)	26	52	3	6.7	<0.00001	1	2.6	<0.00001	2	33.3	0.39
Spitting of collagen + lymphocytic infiltrate (only)	0	0	0	0	NA	0	0	NA	0	0	NA
Spitting of collagen + lymphocytic infiltrate (overall)	23	46	2	4.4	<0.00001	1	2.6	<0.00001	1	16.7	0.17
Mucin deposition + lymphocytic infiltrate (only)	0	0	0	0	NA	0	0	NA	0	0	NA
Mucin deposition + lymphocytic infiltrate (overall)	23	46	2	4.4	<0.00001	1	2.6	<0.00001	1	16.7	0.17
Any one present	30	60	5	11.1	<0.00001	2	5.1	<0.00001	33	50	0.64
Any two present	26	52	б	6.7	<0.00001	1	2.6	<0.00001	2	33.3	0.39
All three present	23	46	2	4.4	<0.00001	1	2.6	<0.00001	1	16.7	0.17
All absent	20	40	40	88.9	<0.00001	37	94.9	<0.00001	3	50	0.64

Table 3: Frequency	of different	histopathological	findings
in patients with TD	(<i>n</i> =30)		

	Number	Percentage
Mucin deposition (only)	0	0
Mucin deposition (overall)	26	86.7
Splitting of collagen (only)	1	3.3
Splitting of collagen (overall)	27	90
Lymphocytic infiltrate (only)	3	10
Lymphocytic infiltrate (overall)	26	86.7
Mucin deposition + splitting of collagen (only)	3	10
Mucin deposition + splitting of collagen (overall)	26	86.7
Splitting of collagen + lymphocytic infiltrate (only)	0	0
Splitting of collagen + lymphocytic infiltrate (overall)	23	76.7
Mucin deposition + lymphocytic infiltrate (only)	0	0
Mucin deposition + lymphocytic infiltrate (overall)	23	76.7
Any one present*	30	100
Any two present ^s	26	86.7
All three present#	23	76.7

*Possible dermopathy; *probable dermopathy; #definite dermopathy

as measured by FT4 level [Table 9], was not related to TD, either. Specificity and PPV for TD in GD were 88.9% and 85.7% for any one histological feature, 93.3% and 89.7% for combination of any two histological features and 95.6% and 92% for all three features. Presence of all the three features had PLR of 10.45 and DOR of 18.31 for TD [Table 10].

DISCUSSION

In the present study, we looked at the histopathological evidence of TD in patients with GD with clinically normal pretibial skin. TAO and TD share common pathogenetic mechanisms with tissue fibroblasts expressing TSH and insulin-like growth factor-1 receptors playing the key role. Histological findings of TD are a combination of deposition of mucinous material (glycosaminoglycan) in the reticular dermis, separation, fragmentation and fraying of collagen fibres and lymphocytic infiltration of the perivascular spaces (superficial dermal vessels), particularly in early lesions.^[10] Accumulation of glycosaminoglycan (hyaluronate) has been suggested as the biochemical hallmark of TD as similar changes in retroocular tissue are found in TAO.[11] However, there perhaps is no pathognomonic feature(s) of TD and several conditions including myxedema of hypothyroidism mimic TD on HPE. Mucinosis associated with stasis dermatitis as seen in patients with venous insufficiency or obesity-associated lymphedematous mucinosis and a few dermatological diseases (chronic or lichenified dermatitis, hypertrophic lichen planus, bullous pemphigoid, papular mucinosis, reticular erythematous mucinosis and follicular mucinosis) often

	With TAO (<i>n</i> =22)		Without TAO (n=28)		Р
	Number	Percentage	Number	Percentage	
Mucin deposition (only)	0	0	0	0	NA
Mucin deposition (overall)	13	59.1	13	46.4	0.37
Splitting of collagen (only)	0	0	1	3.6	0.37
Splitting of collagen (overall)	13	59.1	14	50	0.52
Lymphocytic infiltrate (only)	2	9.1	1	3.6	0.41
Lymphocytic infiltrate (overall)	14	63.6	12	42.9	0.14
Mucin deposition + splitting of collagen (only)	1	4.5	2	7.1	0.70
Mucin deposition + splitting of collagen (overall)	13	59.1	13	46.4	0.37
Splitting of collagen + lymphocytic infiltrate (only)	0	0	0	0	NA
Splitting of collagen + lymphocytic infiltrate (overall)	12	54.5	11	39.3	0.28
Mucin deposition + lymphocytic infiltrate (only)	0	0	0	0	NA
Mucin deposition + lymphocytic infiltrate (overall)	12	54.5	11	39.3	0.28
Any one present*	15	68.2	15	53.6	0.29
Any two present ^s	13	59.1	13	46.4	0.37
All three present [#]	12	54.5	11	39.3	0.28
All three absent	7	31.8	13	46.4	0.29

Table 4: Relation between TAO and histopathological findings in GD (n=50)

*Possible dermopathy; *probable dermopathy; #definite dermopathy

Table 5: Point-biserial correlation coefficient (r) between age and each of the histopathological features in the study population (n=95)

Variables	Cases (n	=50)	Controls ((<i>n</i> =45)
	r	Р	r	Р
Mucin deposition (overall)	-0.1369	0.34	0.1593	0.29
Splitting of collagen (overall)	-0.1765	0.22	0.115	0.45
Lymphocytic infiltrate (overall)	-0.0336	0.82	0.05	0.72

Table 6:	Point-biserial correlation coefficient (r) between
duration	of disease and each of the histopathological
features	in patients with GD $(n=50)$

Variables	Cases (<i>n</i> =50)
	r	Р
Mucin deposition (overall)	0.136	0.34
Splitting of collagen (overall)	0.105	0.47
Lymphocytic infiltrate (overall)	0.068	0.64

Table 7: Comparison of histopathological findings between treatment naïve patients and those on carbimazole (n=50)

Variables	Treatment naïve (n=25)	On carbimazole (<i>n</i> =25)	Р
Mucin deposition (overall)	13 (52%)	13 (52%)	1
Splitting of collagen (overall)	14 (56%)	13 (52%)	0.78
Lymphocytic infiltrate (overall)	14 (56%)	12 (48%)	0.57

demonstrate dermal accumulation of mucin as a prominent feature. Eleven per cent of the control population in our study demonstrated at least one such histological feature and 7% had dermal deposition of mucinous material suggesting that such abnormalities are not specific for TD. Preservation of a zone of normal-appearing collagen in the superficial papillary dermis and mucin deposition in the reticular dermis have been suggested as distinguishing features between pretibial mucinosis of GD from pretibial mucinosis without GD, as both the findings are absent in the latter in a small cohort.^[12] Due to non-specific nature of histopathological alterations, we divided our patients with GD into three groups based on the presence of number of histological features: possible TD, probable TD and definite TD.

TD was diagnosed in 33% of those with autoimmune thyroid disease (AITD) (n = 76) in an earlier study by clinical examination supplemented with sonographic measurement of combined thickness of dermis and subcutaneous tissue and isolated thickness of deeper dermis. Thirty-six per cent of patients with GD in that cohort (n = 58) had TD. Eleven patients (14%) underwent confirmatory skin biopsy of whom five had clinical evidence of dermopathy, three had equivocal signs of dermopathy and the remaining three had normal pretibial skin. Interestingly, one out of these 11 patients with histologically confirmed TD had normal skin thickening, highlighting the fact that TD exists even in patients with normal-appearing pretibial skin.^[5] All the participants in our study had clinically normal pretibial skin and we found at least one histological abnormality (possible TD) in 60% (n = 30), two features (probable TD) in 52% (n = 26) and all three features (definite TD) in 46% (n = 23) of GD, suggesting that true prevalence of TD is much higher. Though we did not perform USG in our cohort, we documented a higher prevalence of TD on HPE compared to those using USG, indicating that USG alone might not be sensitive enough to detect underlying subclinical dermopathy.

The dominant histopathological findings observed in the aforementioned study were marked separation of the collagen fibres (100%), followed by dermal mucin deposition (91%) and lymphocytic infiltrate in the perivascular spaces (72.7%). Combination of separation of collagen fibres and dermal mucin deposition was seen in 91% cases. Splitting of collagen dominated the microscopic features in our study as well (90%), whereas mucin deposition and lymphocytic infiltrate was encountered in 86.7% of patients with TD. Splitting of collagen combined with mucin deposition was evident in 86.7% of our patients. Interestingly, patients with GD without PTM may have histologically proven TD in other sites.^[7] HPE of biopsy specimens obtained from thickened skin over extensor surfaces of the forearms in those patients revealed prominent lymphocytic infiltration around dermal capillaries (77.8%),

Table 8: Point-biserial correlation coefficient (r) between treatment duration and each of the histopathological features in patients with GD treated with carbimazole (n=25)

Variables	Cases on carbir	mazole (<i>n</i> =25)
	r	Р
Mucin deposition (overall)	0.0456	0.75
Splitting of collagen (overall)	0.0209	0.89
Lymphocytic infiltrate (overall)	-0.123	0.39

Table 9: Point-biserial correlation coefficient (r) between FT4 and each of the histopathological features in patients with GD (n=50)

Variables	Cases (<i>n</i> =50)	
	r	Р
Mucin deposition (overall)	-0.041	0.78
Splitting of collagen (overall)	-0.078	0.59
Lymphocytic infiltrate (overall)	-0.118	0.41

distortion of collagen fibres (55.5%) and dense deposits of mucin in the papillary dermis (55.5%).

Prevalence of TAO in GD varies significantly among different studies depending on the diagnostic criteria and ranges from 25% to 84%.^[13,14] Clinical TAO based on the European Group on Graves' Orbitopathy (EUGOGO) recommendations was found in 65 of 235 newly diagnosed patients with GD in India (prevalence 27.6%; 95% confidence interval 22–33%).^[15] Clinically evident TAO was found in 44% of GD in our study.

We documented TD in 54–68% of those with TAO, which is somewhat higher than the earlier studies (31%).^[5] In addition, histological features suggestive of TD were present in 39–54% of those without TAO. TD has often been associated with relatively severe TAO, and TAO is supposed to precede TD. However, PTM has been reported as presenting manifestation of GD in absence of signs and symptoms (both clinical and radiological) of TAO in isolated case reports.^[16-18]

Moreover, the correlation between TD and TAO was discordant in earlier studies.^[5,10,11] Some of them did not observe an increased prevalence of PTM in patients with TAO, either active or inactive. Similarly, our study also failed to establish an association between TAO and any of the histological features, suggesting that temporal association between TAO and TD is not universal, and TD, subclinical in particular, often exists in absence of TAO.

Age, cigarette smoking, local trauma, venous stasis posture, AITD, and TRAb are amongst the identified risk factors of TD with TRAb having the highest odds (odds ratio (OR) 42.93).^[19] Patients with TD often have very high circulatory TRAb. However, biopsy-proven PTM has been reported in individuals with negative thyroid autoantibodies [anti-thyroid peroxidase (TPO), anti-thyroglobulin (Tg) autoantibodies, thyroid-stimulating immunoglobulins and thyroid binding inhibitory immunoglobulins], highlighting the fact that the

Table	10:	Diagnostic	accuracy	of	the	different	histological	features	for	TD	
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Variables	Sensitivity	Specificity	PPV	NPV	PLR	NLR	DOR
Mucin deposition (only)							
Mucin deposition (overall)	52%	93.3%	89.7%	63.6%	7.76	0.51	15.17
Splitting of collagen (only)	2%	97.8%	50%	47.3%	0.91	1.002	0.89
Splitting of collagen (overall)	54%	91.1%	87.1%	64.1%	6.07	0.505	12.03
Lymphocytic infiltrate (only)	6%	97.8%	75%	48.4%	2.73	0.96	2.81
Lymphocytic infiltrate (overall)	52%	93.3%	89.7%	63.6%	7.76	0.51	15.17
Mucin deposition + splitting of collagen (only)	6%	97.8%	75%	48.4%	2.73	0.96	2.81
Mucin deposition + splitting of collagen (overall)	52%	93.3%	89.7%	63.6%	7.76	0.51	15.17
Splitting of collagen + lymphocytic infiltrate (only)							
Splitting of collagen + lymphocytic infiltrate (overall)	46%	95.6%	92%	61.4%	10.45	0.56	18.31
Mucin deposition + lymphocytic infiltrate (only)							
Mucin deposition + lymphocytic infiltrate (overall)	46%	95.6%	92%	61.4%	10.45	0.56	18.31
Any one present	60%	88.9%	85.7%	66.7%	5.41	0.45	12
Any two present	52%	93.3%	89.7%	63.6%	7.76	0.51	15.17
All three present	46%	95.6%	92%	61.4%	10.45	0.56	18.31

pathogenesis of TD is probably multifactorial and yet to be crystallized.^[20-23]

TRAb was estimated in a very small proportion of patients with GD in our study, and we could not estimate the association between TRAb and TD. However, age was not related to any of the three known histopathological findings of TD in our cohort.

It has been postulated that perivascular lymphocytic infiltration appears early in the course of TD. Activated fibroblasts then secrete glycosaminoglycans, a mucin-like substance in extracellular matrix of pretibial tissue, that trap water, thereby leading to compression of dermal lymphatics and non-pitting oedema.^[24] Deposition of mucin leads to separation of dermal collagen. However, we could not establish such sequence of events as duration of disease was not related to the histological findings of TD in our study.

As 4% of our control population also had evidence of definite TD, we calculated the PLR, NLR and DOR, as measures of effectiveness of the three features on HPE as a diagnostic test for TD. PLR denotes the probability of a test being positive in a patient relative to the probability of that test being positive in healthy population. DOR is defined as the ratio of the odds of the test being positive in subjects with disease relative to the odds of the test being positive in subjects without disease. NLR represents the ratio of the probability that a negative result will occur in subjects with the disease to the probability that the same result will occur in subjects without the disease. PLR of more than 1 is used to rule in, whereas NLR of less than 1 is used to rule out the diagnosis. Any test with a PLR of ≥ 10 , DOR ≥ 10 and NLR of ≤ 0.1 is considered an excellent diagnostic test. Presence of all the three histological features had PLR of 10.45 and DOR of 18.31, suggesting HPE can be used as a confirmatory test for TD in appropriate clinical background.

The strength of our study was the large number of punch biopsy specimens examined for histological features of TD. Participants were included only if two separate investigators, unaware of each other's finding, were in favour of clinically normal pretibial skin. The specimens were examined by a single pathologist, blinded about the clinical diagnosis. We, thus, eliminated any possible bias.

Our study had certain limitations. First, we did not estimate any of the thyroid autoantibodies (anti-TPO, anti-Tg or TRAb). PTM has been reported in euthyroid patients without goitre or TAO, but with elevated circulatory thyroid-stimulating immunoglobulin or other thyroid specific autoantibodies.^[25] Although immunofluorescence studies have failed to demonstrate a direct role of these antibodies, TD is presumed to be an autoimmune phenomenon attributable to the presence of such immunoglobulins.^[6] Patients in the control group of our study might have such antibodies in the circulation and confounded the findings in that group. We did not perform orbital imaging and relied on clinical diagnosis of TAO in patients with GD. Patients with GD, who did not have lateral lid flare or visible lower sclera, might have subclinical TAO (thickened EOMs or increased intra-orbital fat) and might have been misclassified. We did not classify the patients with TAO according to severity or activity of the disease. Tobacco smoking is a well-established risk factor for TAO, and an association between tobacco use and PTM has also been suggested. We did not explore such an association in this cohort. In addition to the three histological features assessed in our study, hyperkeratosis of the overlying epidermis, relative absence of hemosiderin and lack of angioplasia may distinguish TD from other dermatological conditions. Though these features were not evaluated in our study, we have documented that combined presence of lymphocytic infiltration, mucin deposition and splitting of collagen accurately confirm TD in majority. We failed to demonstrate significant difference in histopathological features between GD and those with toxic nodular thyroid disease (TMNG/ STN). This was attributable to a very small number of patients in the latter subgroup (n = 6).

CONCLUSION

We conclude that TD is much more prevalent than is thought to be. HPE of pretibial skin has a higher sensitivity for subclinical TD compared to various non-invasive tools like USG. About 46-60% of patients with GD and normal-appearing pretibial skin on naked eye harbour histopathological evidence suggestive of TD. PTM represents an advanced stage of TD and majority of patients with subclinical TD perhaps do not progress to clinically apparent disease; hence, PTM is encountered infrequently. Histopathological evidence of mucin deposition, splitting of collagen fibrils and perivascular lymphocytic infiltrate, though highly suggestive, is not specific for TD as occasional patients without GD or AITD also demonstrate similar abnormalities. Subclinical TD, so, needs to be diagnosed in appropriate settings like thyroid autonomy associated with AITD. Clinically apparent PTM, though is seen exclusively in patients with active or severe TAO, subclinical TD may not be related to clinically diagnosed TAO in GD.

Ethics approval

The study was approved by the Intuitional Ethical Committee of Midnapore Medical College and Hospital. This has been mentioned in the 'MATERIALS AND METHODS' section.

Consent for publication

All the authors provided consent for publication of the study findings and agreed to the authors' list mentioned above. The manuscript has been read and approved by all the authors. Requirements for authorship have been met, and each author believes that the manuscript represents honest work. The corresponding author is responsible for communicating with the other authors about revisions and final approval of the proofs.

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Conflicts of interest

There are no conflicts of interest.

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