ONE YEAR CROSS-SECTIONAL STUDY OF EVALUATION OF ASSOCIATION BETWEEN VITILIGO AND THYROID AUTOIMMUNITY

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LIST OF ABBREVIATIONS:

AA : Adrenal Antibodies

AMA : Anti-Mitochondrial Antibodies

ANA : Antinuclear Antibodies

Anti-Tg : Anti- Thyroglobulin

ATD : Autoimmune thyroid disease.

Anti-TPO Ab : Anti-Thyroid Peroxidase Antibody

COMT : Catechol-O-Methy1 Transferase

CI : Confidence Interval

BH_4 : Tetrahydrobiopterin

GCA : Glucagon Cell Antibodies

GTP : Guanosine Triphosphate

GFRP : GTPCH *(Guanosine Triphosphate Cyclohydrolase)* Feedback Regulatory Protein

ICA : Islet cell antibodies

KP :Koebner's phenomenon

LATS :Long acting thyroid stimulator

MOA :Monoamine Oxidase A

MBEH :Monobenzylether Of Hydropquinone

MA : Melanocyte Antibodies

NSV : Non-Segmental Vitiligo

PCA :Parietal Cell Antibodies

SV : Segmental Vitiligo

SMA : Smooth Antibodies

TRH : Thyrotropin-releasing hormone

TSH : Thyroid Stimulating Hormone

TSA : Tissue Specific Autoantibodies

TMA : Thyroid Microsomal Antibodies

UnV : Undetermined Vitiligo

VASI : Vitiligo Area Severity Index

VGICC :Vitiligo Global Issues Consensus Conference

VIDA : Vitiligo Disease Activity Score

ABSTRACT

Background:

Vitiligo is a common acquired dermatological disorder characterized by depigmented macules and patches. Over the years, researchers have hypothesized many theories for its cause but autoimmune etiology appears most plausible. This is supported by the association of vitiligo with other autoimmune conditions, most commonly thyroid disorders. Vitiligo has been noted to precede thyroid dysfunction in many patients. The assessment of anti-thyroperoxidase (TPO) antibodies in vitiligo patients can be used as a sensitive tool for early detection of subclinical autoimmune thyroid disease.

Aim:

1. To study the relation of vitiligo with demographic data of the participants (like age, sex, duration of lesions, etc.).
2. To evaluate the prevalence of thyroid autoimmunity in vitiligo patients.

Materials and methods:

This was a cross-sectional study conducted at Dr. Prabhakar Kore KLE Hospital and Medical Research Centre, Belgaum from January to December 2012. A total of 100 patients clinically diagnosed (old and new) as having vitiligo were enrolled in the study. Patients with known thyroid disease on supplementation therapy or anti-thyroid medication, those who had undergone thyroid surgery, patients with other causes of leucoderma or those who did not provide informed consent were excluded from the study. Serum anti-TPO antibodies were measured in all the patients.

The serum was considered positive for anti-TPO antibodies if the concentration was greater than 35 IU/ml (Normal range: 0.00 – 35 IU/ml was labelled 'negative'). Detailed profiling (descriptive and comparative) of the study particpants vis-à-vis their anti-TPO antibody status was done using SPSS version 16.0. Non-parametric tests (Mann Whitney U test, Chi-squared test and Fisher's Exact Probability Test) were applied to test statistical significance at a $p < 0.05$.

Results:

The attendance of females was higher than males. Females manifested vitiligo around 10 years earlier than males and this difference was significant (p=0.017). The prevalence of anti-TPO antibody positivity was found to be 28%. We found a significant relationship of anti-TPO Ab with higher mean age (p=0.037) and with presence of coincident precipitating 'trigger' factor (p=0.037) in the participants. Other parameters did not have significant association with anti-TPO positivity.

Conclusion:

According to our study, none of our vitiligo patients had symptoms or signs of thyroid disease at the time of presentation but on biochemical evaluation, anti-TPO antibodies were found in 28% of patients. Hence, we recommend screening of these patients for anti-TPO antibodies, especially in older patients and those giving history of precipitating factors.

Keywords: vitiligo, thyroid autoimmunity, anti-TPO antibodies, anti-TPO positive

TABLE OF CONTENTS

Sl.no.	PARTICULARS	Page no.
1.	**INTRODUCTION**	1
2.	**OBJECTIVES**	2
3.	**REVIEW OF LITERATURE**	3
4.	**MATERIALS AND METHOD**	19
5.	**RESULTS**	22
6.	**DISCUSSION**	58
7.	**CONCLUSION**	72
8.	**SUMMARY**	74
9.	**BIBLIOGRAPHY**	77
10.	**ANNEXURES**	88
	• **Annexure I - Informed consent form**	88
	• **Annexure II - Patient proforma**	92
	• **Annexure III- Photographs**	99
	• **Annexure IV- Master chart**	101
	• **Annexure V- Key to master chart**	105

LIST OF TABLES

Table no.	PARTICULARS	Page no.
i.	New classification for assessment of Koebner's phenomenon proposed by VETF	8
ii.	Bordeaux VGICC classification and consensus nomenclature of vitiligo	11
iii.	Association of vitiligo with other diseases	13
iv.	Comparison of thyroid autoantibody profile in various studies	65
1.	Age characteristics of the study participants	23
2.	Age at onset and duration of the vitiligo lesions in the study sample	25
3.	Association of family history of vitiligo with sex of the participants	26
4.	History of precipitating factors present in the study participants	27
5.	VIDA Score distribution in the study sample	28
6.	Types of vitiligo in the participants	30
7.	Site of onset and current site of involvement in the study participants	32
8.	VASI of the study participants	34

9.	Clinical variants of vitiligo in the study sample	35
10.	Clinical characteristics of vitiligo lesion in the study sample	35
11.	Diseases associated with vitiligo in the study participants	36
12.	Prevalence of Anti - TPO Ab positivity in the study sample	37
13.	Age comparison of study participants according to their Anti-TPO status	38
14.	Association of Anti-TPO Ab status with decadal distribution of the participants	39
15.	Association of Anti-TPO Ab status with sex of the participants	41
16.	Age at onset and duration of the vitiligo lesions according to Anti-TPO Ab status	42
17.	Association of history of vitiligo in family with Anti - TPO Ab status	43
18.	Distribution of history of trigger factors according to Anti-TPO Ab status	44
19.	Distribution of VIDA Scores according to Anti –TPO Ab status	46
20.	Distribution of vitiligo types in the participants according to their Anti-TPO Ab status	47
21.	Site of onset of vitiligo in participants as per their Anti - TPO status	49

22.	Comparison of VASI percentages according to Anti - TPO Ab status	50
23.	Distribution of vitiligo clinical variants according to Anti - TPO Ab status	51
24.	Distribution of Koebner's Phenomenon and Leukotrichia according to Anti-TPO Ab status of participants	52
25.	Comparison of Serum TSH levels according to Anti - TPO Ab status	53
26.	Correlation between Serum TSH levels and Anti-TPO Ab Antibody titres	54
27.	Thyroid status of the participants according to their Anti-TPO Ab status	55
28.	History of associated diseases in study participants according to their Anti - TPO Ab status	57

LIST OF FIGURES

Sl.no.	PARTICULARS	Page no.
1.	Age distribution of the study participants	24
2.	VIDA Scores in the study participants	29
3.	Proportion of vitiligo types in the study sample	31
4.	Sites of onset and current involvement with vitiligo in the study participants	33
5.	Age distribution of the study participants according to their Anti-TPO Ab status	40
6.	Distribution of history of trigger factors according to Anti-TPO Ab	45
7.	Types of vitiligo in the participants (%) according to their Anti-TPO Ab status	48
8.	Percentage distribution of Thyroid Function states in the sample according to the Anti-TPO Ab status of participants	56

LIST OF PHOTOGRAPHS

Sl.no.	PARTICULARS	Page no.
1.	Vitiligo vulgaris	99
2.	Acral vitiligo	99
3.	Segmental vitiligo with leukotrichia	99
4.	Mucosal vitiligo	99
5.	Trichrome vitiligo	100
6.	Halo naevus	100
7.	Koebner's phenomenon in vitiligo	100
8.	Alopecia areata concomitant with vitiligo	100

INTRODUCTION

Vitiligo is defined as a specific, common, often heritable, acquired dermatological disorder characterized by well- circumscribed, milky-white cutaneous macules and patches devoid of identifiable melanocytes.[1]Vitiligo affects 0.1-2% of the world's population. It usually begins in childhood or adolescence, with peak onset at 10 to 30 years, but it may occur at any age. No racial predilection is noted. Both sexes are equally affected.[2]Clinically it can be broadly classified into three major clinical types, segmental, non-segmental and mixed (segmental and non-segmental)[3]

Vitiligo is a multifactorial polygenic disorder with a complex pathogenesis. Although several theories (that include autoimmune, autocytotoxic, biochemical, neural and genetic mechanisms[2]), have been proposed to explain the loss of epidermal melanocytes in vitiligo, the precise cause remains unknown. At present autoimmune theory is most plausible.[4] This is supported by the association of vitiligo with other autoimmune conditions, particularly autoimmune thyroid disorders like Hashimoto thyroiditis and Grave's disease[5,6,7]. These autoimmune thyroid diseases are characterized by elevated serum antibodies directed against thyroid-specific antigens like thyroperoxidase (TPO) and thyroglobulin (Tg). A mean prevalence of 20.8% has been reported in patients with vitiligo for thyroid-specific autoantibodies.[8]

On reviewing the literature we could not find many similar studies in this part of India, hence we were prompted to undertake this study with the objectives mentioned in the following section.

OBJECTIVES

1. To study the relation of vitiligo with demographic data of the participants (like age, sex, duration of lesions, etc).

2. To evaluate the prevalence of thyroid autoimmunity in vitiligo patients.

REVIEW OF LITERATURE

HISTORICAL ASPECT:

The earliest reports on what is known today as vitiligo date back to around 1500 B C; a mention is found in the Eber's Papyrus. References can also be found in the Atharva Veda and Charaka Samhita. Much emphasis on "white spots" can also be found in the Holy Bible and in Greek literature.[9]

GENETICS:

Cockayne described familial aggregation of vitiligo as early as 1933.[10]Many subsequent studies have reports of a high incidence of a positive family history and familial aggregation.[11] Approximately 30% of patients with vitiligo have a family member with vitiligo and up to 21% may have a first degree family member affected.[1] Recent studies that have taken variability of the age of onset into account indicate that vitiligo is controlled by recessive genes at three or four autosomal loci.[12]

PREVALENCE:

Vitiligo has been found to affect 0.1-2% of the world's population, [1]while it accounts for 3 to 4% in India.[13] Reviewers have noted that females have been more commonly reported with vitiligo than males, probably reflecting their concern for cosmetic disfigurement; the frequency in the population is probably the same in both sexes.[14]

ETIOPATHOGENESIS :

The current consensus on the histological presentation of vitiligo is that the

depigmented lesion is devoid of melanocytes.[15]However, the exact etiopathogenesis of vitiligo remains an enigma. Four hypotheses, have been proposed to explain the causation of vitiligo – autoimmune, autocytotoxic, neural and biochemical.[16]

A. Autoimmune hypothesis

A number of indirect observations suggest that vitiligo is an autoimmune disease against pigment cells.[5,6.7,17,18,19]

a. *Antibodies to melanocytes in vitiligo*:

The notion that vitiligo is an autoimmune disease has been supported by the demonstration of specific autoantibodies (referred to as 'vitiligo antibodies') to melanocyte cell surface antigens (referred to as 'vitiligo antigens') in the sera of most patients with vitiligo. They can destroy pigment cells in vitiligo by two different mechanisms - antibody dependent cell mediated cytotoxicity and complement dependent cytotoxicity. The presence of vitiligo antibodies correlates with the activity of the disease and the extent of depigmentation. Their appearance can precede the development of vitiligo.[20]They have been reported in only 50% of cases with minimal vitiligo, but in 93% of those with extensive disease.[21]They can be demonstrated by immunoprecipitation of melanocytes surface antigens, indirect immunofluorescence and ELISA. While antibodies to tyrosinase were initially reported in 61% of cases,[4] subsequent studies have not confirmed this association, and it may merely be an epiphenomenon.[22]

b. Cellular immune responses in vitiligo[4.]

Inflammatory cells, which are markers for the involvement of cellular immune mechanisms, are usually sparse in the lesions of vitiligo and when present they are

most prominent at the periphery of the active lesions. The infiltrate consists of $CD3^{+,}$ $CD4^{+}$ and $CD3^{+}$ T – lymphocytes, and macrophages. It is seen that the cellular immune response does not initiate injury to melanocytes, but only aggravates it, the actual immune mechanism could be a complement dependent process, or an antibody dependent cell-mediated cytotoxicity.

B. Autocytotoxic hypothesis[23]

The autocytotoxic hypothesis is based on the observation that phenol and some of its derivatives are capable of preferentially destroying pigment cells. Various mechanisms for this have been suggested. These include direct toxicity of free-radicals produced from phenolic compounds, a genetic deficiency in the mechanisms for scavenging the toxic precursors of melanin synthesis,[24] and acquired or inherited defects in the membranes of melanosomes that normally block diffusion of toxic by-products of melanin synthesis into the cytoplasm. Other melanin precursors shown to have a cytoxic potential include dopachrome, p-hydroxycinnamic acid and hydroxyphenyl pyruvic acid.

C. Neural pathogenesis[25,26,27,28]

The neural theory is supported by the origin of melanocytes, which are neural crest derivatives. Besides intimate connection of intraepidernal axon terminals with melanocytes has been noted. Studies support the concept of neuronal or neuropeptide (especially neuropetide Y) involvement in the pathogenesis of vitiligo, released by exogenous stimuli (trauma, Koebner's phenomena) or by endogenous stimuli like stress. They have varied functions like inducing melanocyte dendricity and even immunoregulatory effect, suggesting a possible link between the autoimmune hypothesis and the neural theory of vitiligo. Moreover, it has been suggested that

there is an increased release of catecholamines from the autonomic nerve endings surrounding melanocytes in the vitiliginous skin.

D. Biochemical theory[29]

Vitiliginous skin can be distinguished from other leucodermas by the characteristic yellow/green or bluish fluorescence on Wood's lamp examination. Schallreuter et al, attributed this to the accumulation of pteridines (6-biopterin and 7-biopterin) in the vitiliginous skin. The over production of 7-BH_4 in vitiligo patients causes oxidative stress on the melanocytes and decreases the conversion of phenylalanine to tyrosine, resulting in reduced melanin syntheses.

E. Intrinsic (genetic) theory[30,31]

The inheritance of vitiligo does not follow simple Mendelian laws, but shows complex patterns of expressivity and penetrance. Investigators have postulated a polygenic inheritance model involving three unlinked diallielic recessive genes in the expression of vitiligo. Studies have also suggested the concept that some of the genetic factors may manifest as inherent defects in the melanocytes such as aberrant expression of melanocyte – specific proteins.[32]

New insight into pathogenesis of segmental vitiligo[33]:

A 'three-step theory' proposed by N. van Geel et al. in 2012 provides a more integrated view into the current evidence on segmental vitiligo: release of inflammatory cytokines and neuropeptides in presence of trigger factors (step1) →increased antigen presentation or formation of neoantigens (due to enhanced oxidative stress) by a vulnerable subpopulation of melanocytes (step 2)→development of melanocyte-specific T cells leading to immune-mediated destruction or apoptosis (step 3).

CLINICAL FEATURES

The diagnostic lesion of vitiligo is the typical vitiligo macule, which is of variable size, round / oval in shape, has a milky white colour and scalloped margins[1]. There may be one or many macules that tend to enlarge centrifugally overtime.

Age and Sex Predilection:

Vitiligo may appear at any age after birth to senescence. However, the peak age at onset has been reported to be five to thirty years, the average being around twenty years. Some reports have suggested that the mean age of onset is younger in females than in males[3]. However, studies done later have shown no difference in age of onset between the two sexes[10]. While some studies have suggested that females are more commonly affected than males[5,19], more recent studies have shown the prevalence to be the same in both sexes.[12]

Precipitating (trigger) factors[3]:

History of a precipitating factor has been reported in 10 to 76% of cases of vitiligo[34]. Amongst them, physical injury (Koebner's phenomenon) was shown to be the most common followed by emotional stress, systemic illness, sunburn, pregnancy, and parturition. *Koebner's Phenomenon (KP)[35]*is defined as the development of lesions along the lines of specific trauma such as a cut, burn or abrasion. Studies have suggested the incidence to vary from 21-60%, which is higher in childhood than adults (45.5% vs 27.8%). The inciting insult for KP may vary from an obvious abrasion/excoriation to underlying inflammatory dermatoses, to such subtle causes such as distention of skin during pregnancy or even sunburn. KP was more easily elicited adjacent to the lesions suggesting an increased reactivity of the skin at these

sites to trauma. Vitiligo European Task Force group (VETF) proposed a new classification for assessment of Koebner's phenomenon: Type 1, 2 (A + B) and 3.[35]

Table i: New classification for assessment of Koebner's phenomenon proposed by VETF

Koebner subtype	Is considered to be positive....
Type 1	If the answer of the patient is 'yes' or 'sometimes' to the suggested question: 'Did your skin remain white (depigmented) after skin injury during the last year?'
Type 2A	If at clinical inspection depigmentations are present at sites of chronic pressure or repeated friction
Type 2B	If at clinical examination and Wood's light inspection, depigmentations are present and clearly induced by trauma (linear, punctiform, crenate)
Type 3	If at least 1 experimentally induced injury (level 1–3) to the skin induces a clear depigmentation, that remains for several months.

Using the new assessment method for KP, van Geel N et al [36]concluded that in the presence of KP, vitiligo follows a different course, the affected body surface area was significantly higher. Active disease in the last 12 months was more frequently observed while the treatment response was significantly worse. Njoo et al [37] concluded that KP3 may function as a clinical indicator to assess present disease activity and may predict the responsiveness to therapy with topical steroids plus UVA.

Reverse koebnerization is defined as the clearance of an affected skin (i.e. repigmentation) after injury.[38] *Remote reverse Koebner's phenomenon* is the occurrence of spontaneous repigmentation of non-treated lesions in patients with vitiligo undergoing mini-punch grafting[39]. *Satellite repigmentation* is the same phenomenon in the surrounding untreated vitiligo lesions after punch grafting[40].The

pathophysiology hypothesized is that cytokines derived from the donor skin may influence perilesional melanocytes at distant sites after local secretion[39].

***Distribution of lesions**:*

The most frequently involved sites are the face neck, dorsum of the hands, wrists, axillae, umbilicus, nipples, sacrum and the inguinal regions[3] . It may be observed that the common sites are those more frequently subjected to trauma. Mucous membrane (lip, buccal mucosa, gingival and alveolar) involvement is also noted in vitiligo and is common, or rather, easily detectable in dark skinned races. [41,42]It occurs more frequently in males than in females.

***Leukotrichia**:*

Often the follicular melanocytes may be spared while the epidermal melanocytes are destroyed by preferential attack against them. The importance of leukotrichia stems from the fact that it implies depletion of the follicular melanocyte reservoir and subsequent poor response to medical therapy. However presence of leukotrichia is not related to disease activity and does not indicate a rapid downhill course. [43]

Classification of vitiligo[3]:

Vitiligo has been classified in many ways based on the pattern of distribution of lesions, the activity of the disease and the supposed etiology of the disease. Overall a comprehensive and simple classification would be as follows:

Generalized:

- õ Acrofacial vitiligo - Lesions on the acral areas and on the face, very often the perioral areas.
- õ Vitiligo vulgaris - Multiple macules of variable sizes over widely scattered areas often tending to be bilateral and symmetrical.
- õ Lip-tip vitiligo[44] - Lesions affecting the tips of the digits and the lips.
- õ Mixed - Any combination of vitiligo vulgaris and acrofacial vitiligo or vitiligo vulgaris with segmental vitiligo.
- õ Universal vitiligo – This is the term used to describe complete or near complete depigmentation.

Localized:

- Mucosal - Vitiligo affecting mucus membranes of the lips, oral cavity or the genitalia.
- Focal – This consists of one or more macules in one area but not clearly in a segmental or zosteriform distribution.
- Segmental - Number of macules involving a unilateral segment of the body. The lesions stop abruptly at the midline of the affected segment. Lesions distributed in a pseudodermatomal pattern, most commonly affecting the skin innervated by the trigeminal/thoracic/cervical/lumbar/sacral nerves.

  A novel classification of facial segmental vitiligo was put forth by Hann et al in 2000.[45] Five subtypes were proposed, it was later categorized into six subtypes by Kim et al.[46]: Importantly, they noted that segmental vitiligo of the face does not always follow Blaschko's lines, dermatomes or acupuncture lines.

Some authors propose a similar classification based on the pattern of distribution of lesions, the difference being only in the broad subsets being designated as segmental (unilateral), all other forms of vitiligo classified under non-segmental (bilateral) and mixed vitiligo.

The incidence of segmental and non-segmental vitiligo varies in different studies. Most investigators have found vitiligo vulgaris to be the commonest type of non-segmental vitiligo in their case series [51]. While the prevalence of segmental vitiligo from 3.5% to 20.5% of all patients with vitiligo [47].

Koga and Tango[48] in their study have renamed non-segmental and segmental vitiligo as Type A and Type B vitiligo respectively. They have also shown that Type A vitiligo progressed continuously, and is more frequently associated with autoimmune diseases.

More recently Ezzedine et al put forth the revised classification of vitiligo at the Vitiligo Global Issues Consensus Conference (VGICC) 2012 in Bordeaux, France on behalf of the panelists[49].

Table ii: Bordeaux VGICC classification and consensus nomenclature of vitiligo

	Subtypes
Vitiligo/NSV	Acrofacial Mucosal (more than one mucosal site) Generalized Universal Mixed (associated with SV) Rare variants
Segmental Vitiligo	Uni-, bi-, or plurisegmental
Undetermined/ unclassified Vitiligo	Focal Mucosal (one site in isolation)

In addition to the above type of vitiligo, some special forms of vitiligo have been described such as:

Blue vitiligo: Bluish colour of the vitiligo macules appears due to absence of epidermal melanocytes and the presence of numerous melanophages in the skin.[50]

Inflammatory vitiligo: When there is erythema at the margin of a vitiligo macule, it represents the uncommon 'vitiligo with raised inflammatory borders'.

Vitiligo ponctue: This term has been used to describe the occurrence of small confetti tiny hypomelanotic macules occurring on normal skin or a hyperpigmented macule [50]

Course and Progression of Vitiligo:

The exact course of vitiligo is variable and depends on the type of vitiligo, age at onset, presence of various prognostic factors and attempts at therapy[3, 43,51,52]. The natural course of the disease is however one of gradual progression, the lesions increasing both in number and size[3]. *Galloping vitiligo or vitiligo fulminans* is rapid downhill course of vitiligo with extensive depigmentation developing over a few days to weeks. However spontaneous repigmentation has also been reported in about 10-20% of cases of vitiligo[3].

Clinical variants of vitiligo:

The *trichrome* sign or *'vitiligo gradata'* [50], describes a tan coloured zone between the normal skin and the depigmented macules. Some studies have suggested that it could be an isomorphic Koebner's response whereas others suggest that it may indicate that the disease is in the dynamic phase. *Quadrichrome* vitiligo implies the presence of a fourth colour, dark brown at perifollicular sites or marginally in some

cases of repigmenting vitiligo.[1] A case of *pentachrome* vitiligo has also been described, with five shades - black, brown, medium brown, tan and white [53].

ASSOCIATION OF VITILIGO WITH OTHER DISEASES:

The earliest report of vitiligo with disorders of other organ systems was seen with adrenal gland destruction by Thomas Addison (1855)[54]. The various associations with vitiligo have been tabulated below:

Table iii: ASSOCIATION OF VITILIGO WITH OTHER DISEASES

Endocrine Disorders :	*Addison's Disease*[44,55], *Diabetes Mellitus*[18, 56], *Autoimmune Polyglandular Syndromes*[55,57,58]
Haematological Dysfunction :	*Pernicious Anaemia, Autoimmune haemolytic purpura, Idiopathic thrombocytopenic purpura*[19,54,44]
Dermatologic disease :	*Alopecia areata*[59,60], *Psoriasis*[61,62], *Lichen planus*[54], *Atopic dermatitis*[44], *Dermatitis herpetiformis*[38], *Connective tissue disorders*[54], *Chronic actinic dermatitis*[24], *Canities*[24]
Ocular and otic disorders :[63, 64]	*Vogt-Koyanagi-Harada Syndrome, Allezendrani Syndrome*
Chronic infection :[44]	*HIV and Hepatitis C virus infection*

VITILIGO AUTOANTIBODIES AND AUTOIMMUNE DISORDERS:

McGregor, Katz and Doe [65]reported a series of seven patients with vitiligo and pluriglandular insufficiency. The latter was defined as the simultaneous occurrence of two or of the following endocrinopathies – hypothyroidism, diabetes mellitus, pernicious anemia and Addison's disease in association with other conditions like hypoparathyroidism, premature graying of hair, moniliasis and rheumatoid arthritis. The authors proposed autoimmune etiology for these conditions. The vitiligo in this series usually preceded the development of glandular insufficiencies by bedside examination.

Haroulis et al [66] reported a remarkably high incidence (40%) of organ specific autoantibodies in his series of 85 cases of vitiligo. Of the various antibodies the incidence of antithyroid antibodies was the most significant ($p<0.05$).

Grimes et al [67] assayed serum autoantibodies (antinuclear, antithyroid, anti-parietal cell, anti-smooth muscle and anti-mitochondrial) in black patients with vitiligo and an equal number of matched controls and found that vitiligo patients had a statistically significant increase in the frequency of antithyroid autoantibodies as compared to controls (11.4% vs.1.4%).

Mandry et al [68] conducted a study to determine the presence and frequency of organ specific autoantibodies not only in vitiligo patients but also in their first and second-degree relatives and noted a significant increase in the incidence of antithyroid antibodies in the first and second degree relatives of patients as compared to the same in the controls and the general population ($p<0.05$ and 0.001 respectively).

VITILIGO AND THYROID DISEASE

It was Cunliffe et al in 1968[5] who reported the earliest consolidated case series studying the association between vitiligo and thyroid disease. In their study of 56 vitiligo patients, 30% had thyroid disease as compared to 13% in the control group. Anti-thyroglobulin antibodies were present in 25% of vitiligo patients while anti-microsomal antibodies in 11.6% of their cases. The results of this study thus supported the association of vitiligo with thyroid disease.

Ochi et al[69] reported six cases of vitiligo in a series of 90 patients with Grave's disease. They observed a typical pattern of vitiligo involving palms and soles and with minor involvement elsewhere in those cases. Another observation was that in all these cases vitiligo preceded the onset of Graves' disease.

In a study by Betterle et al[70] which assessed the incidence of thyroid antibodies in 3737 subjects without clinical thyroid dysfunction, 31% of the seropositive cases did so later during the study. They suggested, these could be considered as having symptomless autoimmune thyroiditis.

Hegedus et al[71] conducted a study in 35 cases with vitiligo with equal number of control. Fifteen of their patients had one or more signs of thyroid disease while nine patients had elevated levels of anti-TPO antibodies in their serum. This study thus corroborates the fact that patients of vitiligo seem to run an increased risk of developing autoimmune thyroid disease.

An Indian study by Dave and colleagues[72] showed that no vitiligo patient was found to have thyroid disease clinically; however, on assays, thyroid abnormality (endocrine, immunological or both) was found in 57.1% of the cases as against 10%

of the controls ($p<0.05$). The prevalence of thyroid antibody positivity was found to be 31.4% of vitiligo cases as against 10% of controls ($p<0.05$). They stressed that patients with vitiligo should be screened for thyroid dysfunction, especially those with mucosal vitiligo as 35% of them had thyroid dysfunction as against 6.7% in those without ($p<0.05$).

In another Indian study by Altaf H et al[73] on 192 vitiligo patients, 84% were found to be euthyroid, 15% were hypothyroid and 1% were hyperthyroid. Anti-TPO antibodies were present in significant number of vitiligo patients (11%). The association of anti-TPO antibody was statistically significant with vitiligo ($p = 0.001$) and thyroid profile of the patient ($p < 0.0001$).

Nunes DH et al[74] studied 85 patients out of which autoimmune thyroid diseases were found in 22.4%. Of these, 80% were hypothyroid, 13.3% were hyperthyroid and 6.7% had subclinical hypothyroidism. Five patients presented hypothyroidism prior to the lesions of vitiligo. All the patients with positive anti-Tg Ab also had positive anti-TPO Ab. The unique finding in their study was that patients with positive thyroid autoantibodies showed a probability of extension of vitiligo greater than 25%.

Ninety-four cases of vitiligo were enrolled in a case control study by Maryam D et al.[75] Anti-TPO Ab were detected in 18.1% of patients affected by vitiligo, while this figure was 7.3% in the control group; the difference was significant with p-value < 0.025. Their study showed older cases were at higher risk of evolution toward subclinical thyroid diseases.[76]

Not many studies have stressed on the association of presence of thyroid antibodies in children affected with vitiligo. More recently a prospective

observational study on 114 children with vitiligo was done by Mazereeuw-Hautier J et al in 2010[77], where a second examination was performed one year after inclusion and the clinical characteristics of segmental vitiligo were compared. No child had clinical signs or symptoms of thyroid disease at presentation. On biochemical evaluation thyroid abnormalities were found only in 11.23% with non-segmental vitiligo versus none of the children with segmental vitiligo (P = 0.0001). Of the 10 patients with thyroid abnormalities, six (all girls) had hypothyroidism with positive anti-thyroglobulin or anti-thyroperoxidase antibodies. The remaining four patients (three girls, one boy) had positive antibody titres only.

Adel A. Imam et al[78] conducted a case control study in an Egyptian population in sixty vitiligo patients. Results of their study revealed that serum level of anti-TPO Ab was significantly elevated in vitiligo patients (27%) compared to healthy controls (7.5%). In addition, there was a positive significant correlation between the serum level of anti-TPO Ab and the prevalence of autoimmune thyroid disease. A higher percentage of patients in the adolescence (40%) and early adulthood age groups (33.3%) had elevated anti-TPO Ab compared to children (13.3%). A significantly higher prevalence of elevated serum anti-TPO Ab was detected among female cases (36.7%) compared to male cases (16.7%). Hence, they recommended that periodical estimation of serum anti-TPO Ab level and scanning for autoimmune thyroid disease, especially in adolescent female vitiligo patients.

SB Cho et al[79] undertook a retrospective analysis of 324 Korean children and adolescents aged equal to or less than 20 years. Four had Hashimoto's thyroiditis, two had Graves' disease and seven had subclinical hypothyroidism. Female patients had a significantly higher frequency of associated diseases than male patients (P = 0.024).

In contrast to above studies in children with vitiligo, they concluded no significant association of thyroid disease between children and adolescents with vitiligo, and the control group.

C. Vrijman et al in 2012[8] put forth a systematic review of the prevalence of thyroid disease in patients with vitiligo by analyzing forty-eight studies published between 1968 and 2012 which met their inclusion criteria. They found that thyroid disease, autoimmune thyroid disease and presence of thyroid-specific autoantibodies showed a mean prevalence of 15.1%, 14.3% and 20.8% in patients respectively, with vitiligo and a risk ratio of 1.9, 2.5 and 5.2 respectively, (all statistically significant) compared with patients without vitiligo. This review showed an increased prevalence and an increased risk of autoimmune thyroid disease in patients with vitiligo compared with non-vitiligo which seems to increase with age. The median prevalence of autoimmune thyroid disease is almost thrice as low in children (6.89%) compared with adults (18.6%). They support the recommendation that elevated thyroid antibodies may serve as a useful tool to screen for autoimmune thyroid disease as they can be present up to seven years before clinical diagnosis[80,81]. For this they recommended measurement of anti-TPO antibodies rather than anti-Tg antibodies for screening in euthyroid patients with vitiligo as it is established as a sensitive tool for the detection of early subclinical autoimmune thyroid disease[76] and identification of cases at risk for the same; besides anti-TPO antibodies tend to have more correlation with thyroid dysfunction than anti-Tg antibodies[82].

MATERIAL AND METHODS

A cross-sectional study design is suited for estimating prevalence of disease and traits. It can be used to describe participants' attributes as well. However, comparisons can be made between individuals with the disease/trait and those without. We adopted this ***analytical cross-sectional design*** for this study. The details of the study methodology are described below:

(a) ***Study site:*** The study was conducted in the Dermatology OPD at KLE's Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum as a part of the MD academic curriculum.

(b) ***Study duration:*** The study synopsis was submitted to the Institutional Review Committee in 2011 following which data collection was undertaken in 2012.

(c) ***Ethical clearance:*** It was granted by the J.N.M.C. Institutional Ethics Committee on Human Subjects Research, 2011-12.

(d) ***Sampling frame:*** Patients attending the Dermatology OPD at KLE's Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum between January to December 2012, who were clinically diagnosed (old and new) as having vitiligo and willing to provide written informed consent, were to be considered for inclusion in the study.

(e) ***Selection criteria:*** Patients with known thyroid disease on supplementation therapy or anti-thyroid medication, those who had undergone thyroid surgery, patients with other causes of leucoderma or those who did not provide informed consent were excluded from the study.

(f) ***Sample size :*** Considering the formula

$n = (z^2 \times p \times q)\ d^2 = 4pq/d^2$

Where,

p is prevalence = 31.4% [72]

q is 100-p = 100-31.4= 68.6

d is absolute error ie 10 %

z for 95 % confidence is 1.96 ~ 2

Thus,

n=4x31.4x68.6/(10X10)

=86.16 ~ 87

We included 15 (17.7%) more participants to avoid data insufficiency due to any inadvertent data loss during study conduct. Thus, the total sample size for the study was 100.

(g) ***Sampling techniques:*** Consecutive patients satisfying the selection criteria were included in study till the requisite sample size was met.

(h) ***Data collection:*** A structured proforma was designed to collect information from the study participants. This proforma was pretested in the Dermatology OPD (where the study was later conducted) and finalised. Detailed medical history taking and physical examination were undertaken to make a clinical diagnosis of vitiligo, and the same were documented in the proforma. Venous blood was collected from the subjects and transported to the hospital's central laboratory where it was processed to assess serum thyroid stimulating hormone and anti-thyroid peroxidase antibodies level. Serum TSH was quantitatively measured by

quantitative chemiluminescent immunoassay using a commercially available kit on Advia Centaur XP (Siemens). A diagnosis of hypothyroidism was made when the thyroid function test showed raised serum TSH levels; hyperthyroidism was diagnosed if associated with depressed levels of TSH; normal range of Serum TSH being 0.49-4.67 µIU/ml. Anti-thyroid peroxidase antibodies were assayed by quantitative chemiluminescent immunoassay on Advia Immulite 1000 (Siemens). The serum was considered positive for antithyroid peroxidase antibodies if the concentration was greater than 35 IU/ml (Normal range: 0.00–35 IU/ml was labelled 'negative').

(i) ***Data analysis:*** Data from the proforma was entered into Microsoft Office 2013 Excel spreadsheet. Detailed profiling (descriptive and comparative) of the study particpants vis-à-vis their anti-TPO antibody status was done using SPSS version 16.0. Non-parametric tests (Mann Whitney U test, Chi-squared test and Fisher's Exact Probability Test) were applied to test statistical significance at a $p < 0.05$.

(j) ***Representation of data:*** Data was represented in contingency tables in the form of absolute numbers and proportions. Results from tests for significance were enumerated in the tables wherever applicable. Graphical representation were made using pie and bar diagrams.

RESULTS

The present one year cross-sectional study was conducted in the Department of Dermatology, Venereology and Leprosy of KLE's Dr Prabhakar Kore Hospital and Medical Research Centre, Belgaum. A total of 100 patients with vitiligo who satisfied the selection criteria were included in the study. The data collected from these participants was analysed and the results are described below:

(a) Description of the study sample characteristics [relation of vitiligo with demographic data of the participants (like age, sex, duration of lesions, etc.)]

(for Objective 1) **: Tables 1-11**

(b) Evaluation of the prevalence of thyroid autoimmunity in vitiligo patients

(for Objective 2) **: Tables 12-28**

(a) Description of the study sample characteristics [relation of vitiligo with demographic data of the participants (like age, sex, duration of lesions, etc.)] ***(for Objective 1)*** **: Tables 1-11**

Table 1: Age and sex characteristics of the study participants (N=100)

Sl. No.	Age group (years)	Sex [No. (%)]*		Total
		Male	Female	
1	0-9	03 (07.3)	04 (06.8)	07 (07.0)
2	10-19	07 (17.1)	14 (23.7)	21 (21.0)
3	20-29	06 (14.6)	19 (32.2)	25 (25.0)
4	30-39	06 (14.6)	10 (16.9)	16 (16.0)
5	40-49	11 (26.8)	10 (16.9)	21 (21.0)
6	50 & Above	08 (19.5)	02 (03.4)	10 (10.0)
Total		41 (100.0)	59 (100.0)	100 (100.0)
Mean Age±SD (Median) in Years		33.88+15.86 (35.00)	26.44±13.70 (24.00)	29.49±15.00 (28.00)

*Mann Whitney U test of significance applied between male and female participants: p=0.017

Inference: Majority of the males were past 30 years of age while the females were mostly under thirty. The mean age of the study participants was around 30 years with a standard deviation of 15 years. The males were relatively older than the females. The age difference for the sexes was statistically significant (p=0.017)

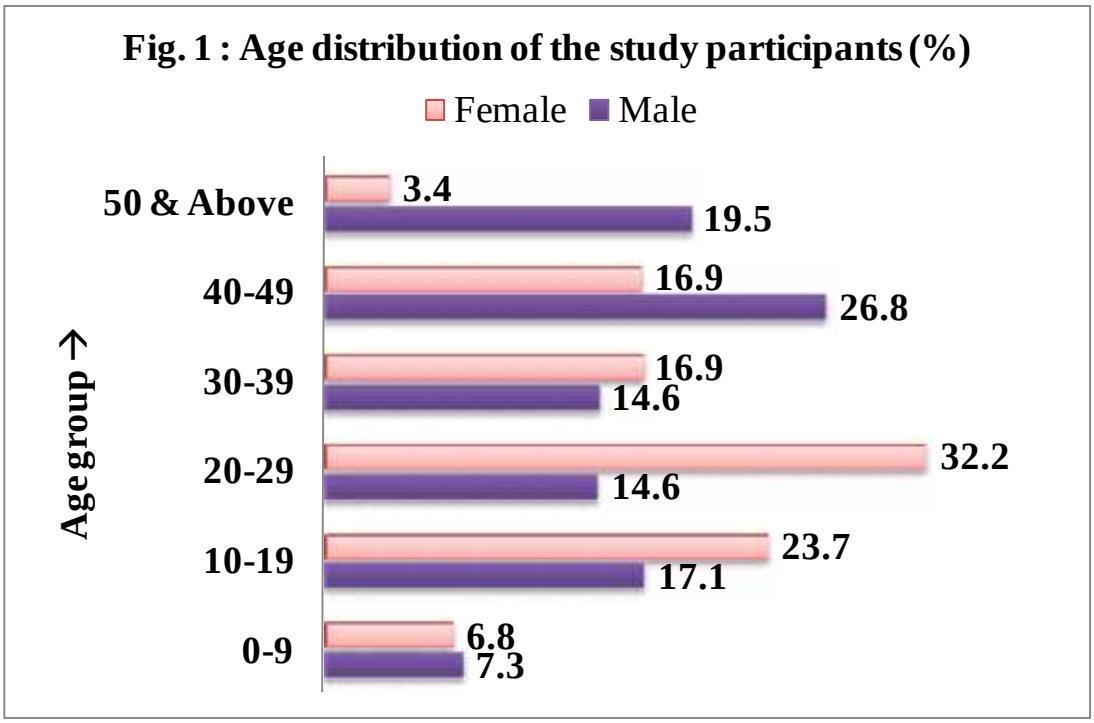
Fig. 1 : Age distribution of the study participants (%)
Female
Male
Age group →
50 & Above
40-49
30-39
20-29
10-19
0-9
3.4
19.5
16.9
26.8
16.9
14.6
32.2
14.6
23.7
17.1
6.8
7.3

Table 2: Age at onset and duration of the vitiligo lesions in the study sample (N=100)					
Sl. No.	**Characteristics of vitiligo lesions**	**Mean±SD (Median) in years**			**P value***
		Overall (N=100)	**Males (N=41)**	**Females (N=59)**	
1	Age at onset	24.80±15.66 (21.25)	30.43±15.88 (31.75)	20.89±14.38 (17.00)	0.004
2	Duration	4.64±6.05 (2.00)	4.31±8.51 (2.00)	5.55±5.95 (2.00)	0.185
* Mann Whitney U test of significance applied between male and female participants.					

Inference: The mean age at onset of vitiligo lesions in the study participants was 24.8 years. Females had an onset almost a decade before males. The p value of 0.004 showed that the difference was statistically significant.

The study sample had vitiligo lesions with a mean duration of 4.64 years. There was no significant difference between males and females regarding this.

Table 3: Association of family history of vitiligo with sex of the participants (N=100)				
Sl. No.	**History of vitiligo in family**	**Sex [No. (%)]**		**Total [No.(%)]**
		Male	**Female**	
1	Present	03 (07.3)	10 (16.9)	13 (13.0)
2	Absent	38 (92.7)	49 (83.1)	87 (87.0)
	Total	41 (100.0)	59 (100.0)	100 (100.0)
Fisher's exact probability=0.229				

Inference: A positive family history of vitiligo was elicited in 13% of the study participants. This was not significantly associated with the sex of the participant (p=0.229).

Table 4: History of precipitating factors for vitiligo present in the study participants (N=100)		
Sl No.	**Precipitating factors**	**Frequency [No. & %]**
1	Physical Trauma	03
2	Emotional stress	02
3	Physical stress	01
4	Sun exposure	01
5	Pregnancy	01
Total		08

Inference: Precipitating factors were reported by eight percent of the participants. The most common factor reported was physical trauma (three percentage).

Table 5: Vitiligo Disease Activity (VIDA) Score distribution in the study sample (N=100)		
Sl. No.	**VIDA Score**	**No. & %**
1	Minus One (-1)	01
2	Zero (1)	14
3	One (2)	55
4	Two (3)	17
5	Three (4)	11
6	Four (5)	02
Total		**100**

Inference: Fifteen percent of the study participants had a stable vitiligo disease as evident from their VIDA Scores of '≤0'. Of the remaining 85 participants, 55 had a score of '1'.

Fig. 2: VIDA Scores in the study participants (N=100)

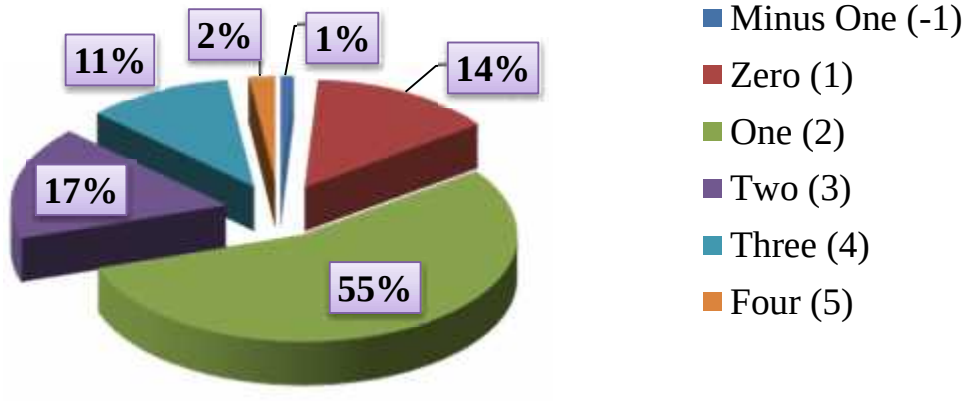

Table 6: Types of vitiligo in the study participants (N=100)		
Sl. No.	**Vitiligo Type**	**No. & %**
1	**Segmental**	04
2	**Non-segmental**	96
	Acral	*05*
	Acrofacial	*02*
	Focal	*12*
	Mucosal	*06*
	Vulgaris	*71*
Total		**100**

Inference: Majority (96%) of the participants had non-segmental type of vitiligo, the most common being vitiligo vulgaris (71 patients). Only four patients had segmental vitiligo.

Fig. 3: Proportion of vitiligo types in the study sample (N=100)

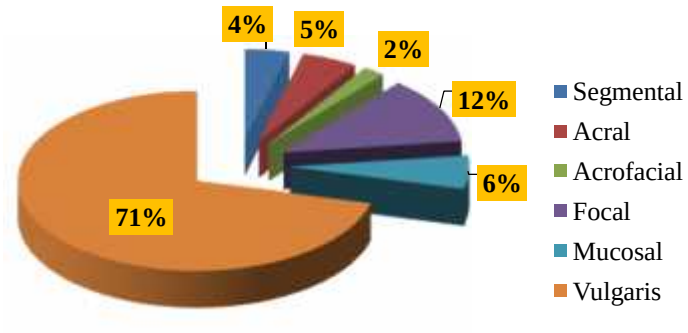

Table 7: Site of onset of vitiligo lesions and current site of involvement in the study participants (N=100)

Sl. No.	Site	Involvement at Onset (No. & %)	Current involvement (No & %)*
1	Head & Neck	22	42
2	Trunk	22	42
3	Genitals	02	09
4	Upper Limb	20	52
5	Lower Limb	34	75
6	Mucosa	-	17

*More than one site was involved in most participants

Inference: Vitiligo onset was most common in the lower limb. The lower limb was also the most common site of current involvement of the lesions in the study participants.

Fig. 4: Sites of onset and current involvement with vitiligo in the study participants (N=100)

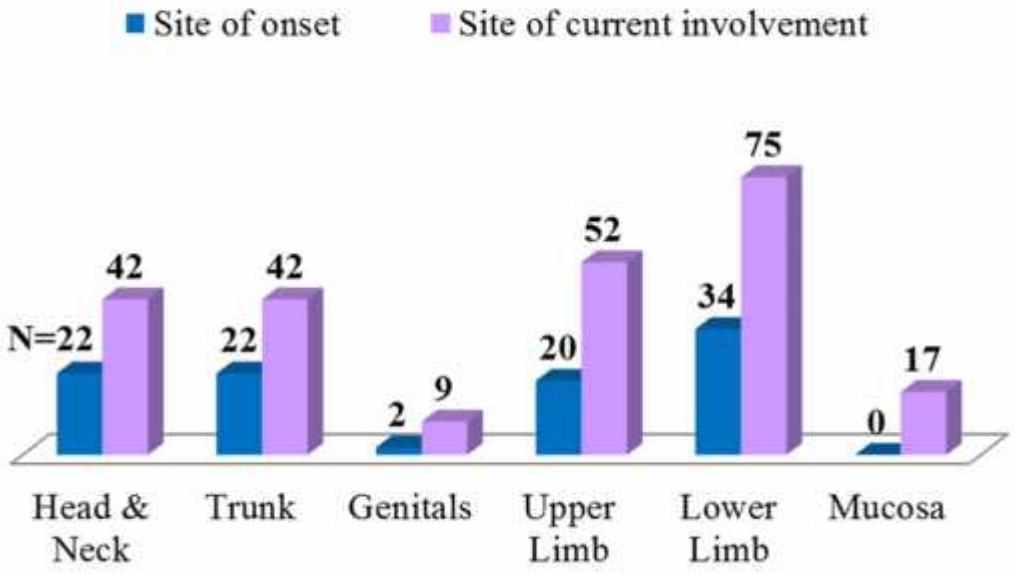

Table 8 : Vitiligo Assessment Severity Index (VASI) of the study participants (N=100)

Sl. No.	VASI %	No. & %
1	0-0.99	22
2	1-4.99	60
3	5-9.99	15
4	10 & above	03
Total		**100**

Inference: Sixty percent of the study participants had a VASI % score between 1 to 4.99. A score of 'less than 1' was found in 22 participants.

Table 9: Clinical variants of vitiligo in the study sample (N=100)		
Sl. No.	**Variant**	**No. & %**
1	Quadrichrome	06
2	Trichrome	06
3	Could not be specified	88
	Total	100

Inference: Quadrichrome and Trichrome variants of vitiligo were seen in six participants, each. No specific variant could be diagnosed for the remaining 88 patients.

Table 10: Clinical characteristics of vitiligo lesion in the study sample (N=100)		
Sl. No.	**Variant**	**No. & %**
1	Koebner's phenomenon	05
2	Leucotrichia	14*
*One participant had associated Halo naevus		

Inference: Of the 100 participants, Koebner's phenomenon was seen in five. Leucotrichia was found in 14 participants which included one with halo naevus as well.

Table 11: Diseases associated with vitiligo in the study participants (N=100)			
Sl. No.	**Disease group**	**Diagnosis**	**No. & %**
1	**Dermatological**	Aphthous stomatitis with xerosis	01
		Irritant contact dermatitis	01
		Congenital melanonychia	01
		Nevus acromicus	01
		Subacute eczema	01
		Total	**05**
2	**Systemic**	Diabetes Mellitus	03
		Diabetes Mellitus with Bronchial Asthma	01
		Total	**04**
3	**Autoimmune (non- thyroid)**	*Duodenitis/gastritis with pernicious anemia*	01
		Alopecia areata	01
		Total	**02**

Inference: Associated dermatological diseases were found in five percent of the participants Among systemic diseases, Diabetes Mellitus (DM) was seen in three patients while another patient had both DM and Bronchial Asthma. Among autoimmune diseases (non-thyroid), gastritis and pernicious anaemia, and alopecia areata were seen in one patient each.

Evaluation of the prevalence of thyroid autoimmunity in vitiligo patients ***(for Objective 2)*** **: Tables 12-28**

Table 12: Prevalence of Anti - TPO Ab positivity in the study sample (N=100)		
Serum Anti - TPO Ab status	**No.**	**%**
Positive (>35IU/ml)	28	28.0
Negative (≤35 IU/ml)	72	72.0
Total	100	100.0

Inference: The upper limit of the anti- TPO Ab was as per the hospital laboratory reference range according to which the participants were labelled Anti - TPO Ab positive (serum titre >35IU/ml). Of the 100 participants with vitiligo, 28 tested Anti - TPO positive. The remaining 72 had a negative Anti - TPO Ab titre (Normal range : 0-35 IU/ml)

Table 13: Age comparison of study participants according to their anti-TPO status (N=100)					
Anti-TPO Ab Group	**No.**	**Mean**	**Std. Deviation**	**Mann-Whitney U**	**P value**
Positive	28	34.50	13.99	737.0	0.037
Negative	72	27.54	15.02		

Inference: The mean age of participants in the Anti-TPO Antibody positive group was 34.5 years as compared to 27.5 years in the antibody negative group. The standard deviations in both the group showed a skewing of the age distribution. Non-parametric comparison (Mann Whitney U test) found a significant difference (p=0.037).

Table 14: Association of Anti-TPO Ab status with decadal distribution of the participants (N=100)

Sl. No.	Age group (years)	Anti-TPO Antibody Status	
		Positive No. (%)	Negative No. (%)
1	0-9	01 (03.6)	06 (08.3)
2	10-19	02 (07.1)	19 (26.4)
3	20-29	09 (32.1)	16 (22.2)
4	30-39	04 (14.3)	12 (16.7)
5	40-49	09 (32.1)	12 (16.7)
6	50 & Above	03 (10.7)	07 (09.7)
Total		28 (100.0)	72 (100.0)

Inference: Participants in the anti-TPO Ab positive group were mostly in their third decade and beyond. Those in the anti-TPO negative group were mostly in their second decade of life while representation consistently decreased in the subsequent decadal age bands.

Fig.5: Age distribution of the study participants according to their Anti-TPO Ab status (%)

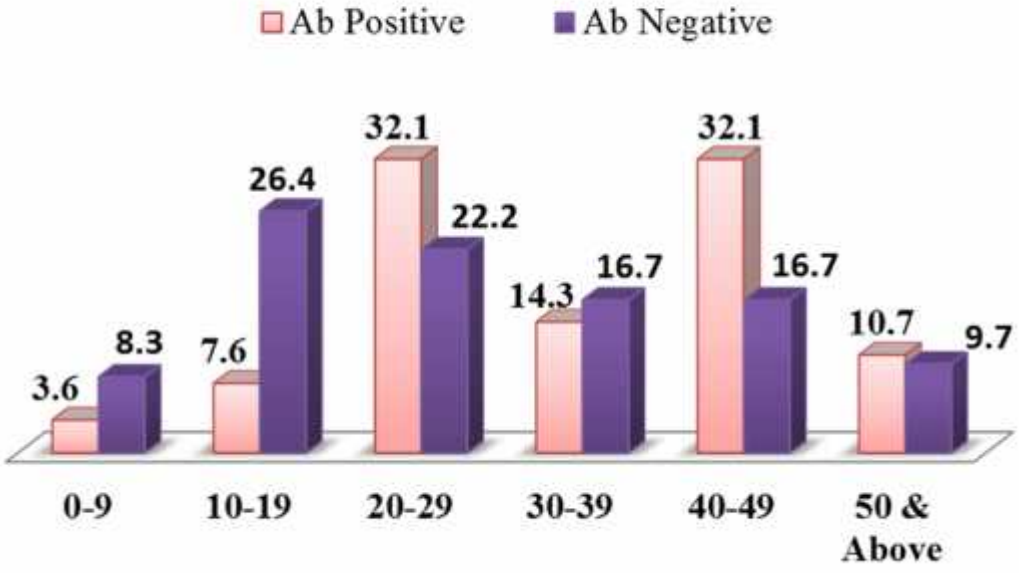

Table 15: Association of anti-TPO Ab status with sex of the participants (N=100)

Sex	Anti - TPO Ab Status	
	Positive **No. (%)**	**Negative** **No. (%)**
Female	20 (71.4)	39 (54.2)
Male	08 (28.6)	33 (45.8)
Total	28 (100.0)	72 (100.0)
$\chi 2=2.483$, Df=1, p=0.115		

Inference: The proportion of females in the Anti-TPO Ab positive group was higher (71.4%) than in the Anti-TPO Ab negative group (54.2%). It was reverse for the males. Statistically, this association was not significant (p=0.115).

Table 16: Age at onset and duration of the vitiligo lesions according to Anti-TPO Ab status (N=100)

Sl. No.	Characteristics of vitiligo lesions	Anti - TPO Ab Status		Mann-Whitney U	P value*
		Positive (N=28) Mean±SD	Negative (N=72) Mean±SD		
1	Age at onset	24.59±15.46	24.88±15.83	1005.00	0.982
2	Duration	5.19±6.63	4.42±5.85	958.00	0.700

* Mann Whitney U test of significance applied between male and female participants

Inference: The age at onset of the vitiligo lesions showed no significant difference according to the Anti-TPO Ab status of the participants (p=0.982). The mean age at onset in either groups was around 25 years.

Those participants testing positive for anti-TPO antibodies, reported a longer duration of vitiligo as compared to those who tested negative. However, this was not statistically different (p=0.7). The mean duration of vitiligo in the former group was around five years as compared to 4.4 years in the latter.

Table 17 : Association of history of vitiligo in family with anti-TPO Ab status (N=100)		
H/O vitiligo in family	**Anti - TPO Ab Status**	
	Positive No. (%)	**Negative No. (%)**
Present	03 (10.7)	10 (13.9)
Absent	25 (89.3)	62 (86.1)
Total	28 (100.0)	72 (100.0)
Fisher's exact probability = 1.000		

Inference: A positive family history of vitiligo was found in 10.7% of the anti-TPO Ab positive participants as compared to 13.9% in those found negative. The association was not significant.

Table 18 : Distribution of history of trigger factors according to Anti-TPO Ab status (N=100)

Sl. No.	Triggering Factors	Anti - TPO Ab Status	
		Positive No. (%)	**Negative No. (%)**
1	Emotional Stress	0 (0)	02 (02.8)
2	Physical Stress	01 (03.6)	0 (0)
3	Pregnancy	01 (03.6)	0 (0)
4	Sun exposure	0 (0)	01 (01.4)
5	Physical Trauma	03 (10.7)	0 (0)
6	None	23 (82.1)	69 (95.8)
Total		28 (100.0)	72 (100.0)

*Fisher's exact probability = 0.037 for *'triggers present'* versus *'triggers absent'*

Inference: A history of coincident triggering factor with vitiligo was elicited more commonly with those with Anti-TPO Ab as compared to those Anti-TPO Ab negative (p=0.037). Physical trauma was the most common trigger of vitiligo in the Anti-TPO Ab positive participants.

Fig. 6: History of trigger factors according to Anti-TPO Ab status of the study participants (N=100)

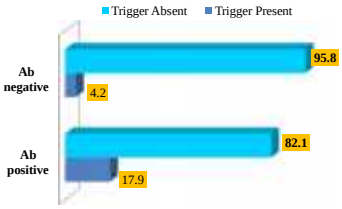

Table 19: Distribution of VIDA Scores according to Anti –TPO Ab status (N=100)

VIDA Score	Anti - TPO Ab Status	
	Positive No.(%)	**Negative No.(%)**
-1	01 (03.6)	0 (0)
0	0 (0)	14 (19.4)
1	20 (71.4)	35 (48.6)
2	03 (10.7)	14 (19.4)
3	04 (14.3)	07 (09.7)
4	0 (0)	02 (02.8)
Total	28 (100.0)	72 (100.0)
P=0.222 for VIDA $\leq$0 &$\geq$1		

Inference: A VIDA Score of 1 accounted for the majority irrespective of the Anti - TPO Ab status. However, 19.4% of the Anti - TPO Ab negative participants had a score of zero or less than zero as compared to only 3.6% of those found positive. More than 96% of Anti-TPO Ab positive and 80.6% of the Anti-TPO Ab negative participants had unstable vitiligo (VIDA$\geq$1). No significant difference was found (p=0.222).

Table 20: Distribution of vitiligo types in the participants according to their Anti-TPO Ab status (N=100)

Sl. No.	Vitiligo Type	Anti-TPO Ab Status	
		Positive No. (%)	**Negative No. (%)**
1	Segmental	01 (03.6)	03 (04.2)
2	Non-segmental	27 (96.4)	69 (95.8)
	Acral	03 (10.7)	02 (02.8)
	Acrofacial	01 (03.6)	01 (01.4)
	Focal	03 (10.7)	09 (12.5)
	Mucosal	01 (03.6)	05 (06.9)
	Vulgaris	19 (67.9)	52 (72.2)
Total		28 (100.0)	72 (100.0)

*Fisher's exact probability (segmental versus non-segmental types) =1.000

Inference: Vitiligo vulgaris accounted for the majority among Anti-TPO positive as well as negative participants. Focal vitiligo was the next common variant. Segmental vitiligo was found in only one patient with TPO positivity and three patients with TPO negative status. No significant association was found for segmental and non-segmental vitiligo in the two groups (p=1.000).

Fig.7: Types of vitiligo in the participants (%) according to their Anti-TPO Antibody status (N=100)

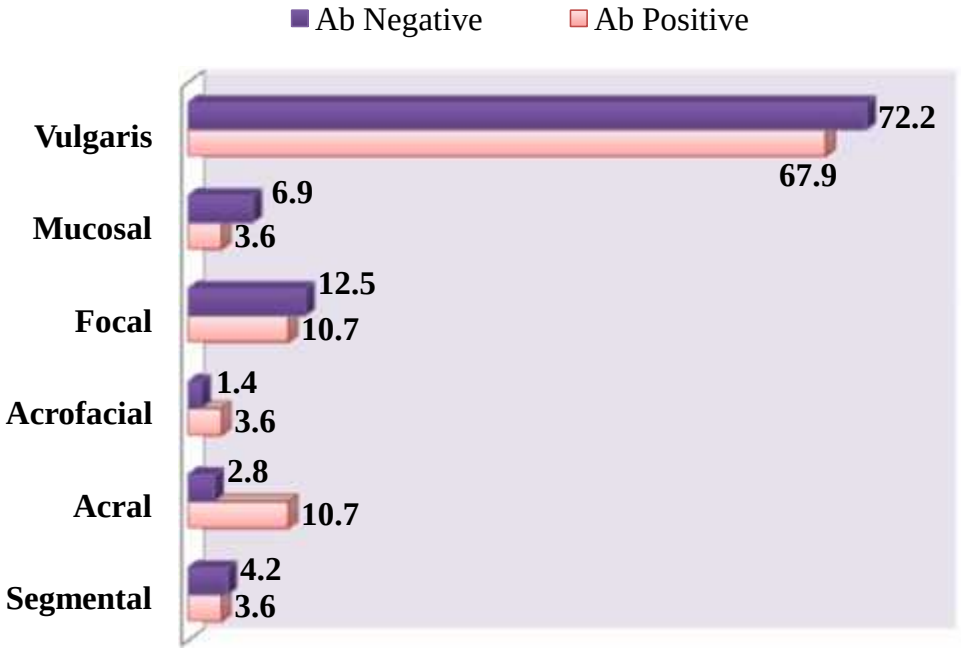

Table 21: Site of onset of vitiligo in participants as per their Anti-TPO Ab status (N=100)			
Sl. No.	**Site of onset**	**Anti-TPO Ab Status**	
		Positive No. (%)	**Negative No. (%)**
1	Head & Neck	07 (25.0)	15 (20.8)
2	Trunk	07 (25.0)	15 (20.8)
3	Genitals	0	02 (02.8)
4	Upper Limb	07 (25.0)	13 (18.1)
5	Lower Limb	07 (25.0)	27 (37.5)
Total		28 (100.0)	72 (100.0)

Inference: While equal number of participants in the anti-TPO Ab positive group reported site of onset of vitiligo in head and neck, trunk, upper limb and lower limb (25% each), none reported genitals as a site of onset. In the anti-TPO Ab negative group, majority had onset of vitiligo in the lower limb (37.5%) followed by head and neck and trunk regions (20.8% each).

Table 22: VASI percentages of the study participants according to their anti-TPO status (N=100)

VASI Score	Anti - TPO Ab status	
	Positive (%)	**Negative (%)**
1	09 (32.1)	13 (18.1)
2	16 (57.1)	44 (61.1)
3	03 (10.7)	12 (16.7)
4	0	03 (04.2)
Total	**28 (100.0)**	**72 (100.0)**

Inference: Majority of the Anti-TPO Ab positive and negative participants had a VASI score of '2'. The mean VASI percentages in the anti-TPO positive group was 2.40 and negative was 3.12 but it did not have a significant difference (p=0.376).

Table 23: Distribution of vitiligo clinical variants according to Anti - TPO Ab status (N=100)

Sl No.	Vitiligo clinical variant	Anti - TPO Ab status	
		Positive No.(%)	**Negative No.(%)**
1	Quadrichrome	02 (07.1)	04 (05.6)
2	Trichrome	03 (10.7)	03 (04.2)
3	None	23 (82.1)	65 (90.3)
Total		28 (100.0)	72 (100.0)

Inference: While nearly 10% of the participants with Anti - TPO Ab positivity had the trichrome variant of vitiligo, the proportion was just 4.2% for their Anti-TPO Ab negative counter-parts. Similarly around 7% of the participants with Anti-TPO Ab positivity had the quadrichrome variant of vitiligo, the proportion was 5.2 % for their Anti-TPO Ab negative counter-parts. Majority (82.1% and 90.3% participants in these respective groups) could not be identified with any particular variant.

Table 24: Distribution of Koebner's Phenomenon and Leukotrichia according to Anti-TPO Ab status of participants (N=100)

Feature	Anti - TPO Ab Status		P value (Fisher's Exact)
	Positive (%) (N=28)	Negative (%) (N=72)	
Koebner phenomenon	03 (10.7)	02 (02.8)	0.132
Leukotrichia	04 (14.3)	10 (13.9)	1.000

Inference: Koebnerization was more frequently reported in Anti - TPO Ab positive participants (10.7%) as compared to the Anti - TPO Ab negative ones (2.8%). The p value of 0.132 was not significant.

Leukotrichia was equally frequent in both the groups (around 14% in both).

Table 25: Comparison of Serum TSH levels according to Anti - TPO Ab status (N=100)				
Sl. No.	**Anti - TPO Ab status**	**No.**	**Serum TSH level (µIU/ml)**	
			Mean	**Std. Deviation**
1	Positive	28	11.31	41.03
2	Negative	72	2.67	2.27
Mann Whitney U = 923.000, P = 0.514				

Inference: The serum TSH levels in either group showed a skewed distribution with no significant difference (p=0.514). However, the mean TSH level was almost five times in Anti - TPO Ab positive participants than the Anti - TPO Ab negative subjects.

Table 26 : Correlation between Serum TSH levels and Anti-TPO Ab Antibody titres (N=100)				
Biochemistry	Mean	SD	Spearman's rho	P value
Serum TSH	5.09	21.87	0.052	0.609
Serum Anti-TPO Ab titre	75.41	206.77		

Inference: A correlation coefficient of 0.052 proved that serum TSH and Serum Anti-Ab titre were mutually unrelated (p=0.609).

Table 27 :Thyroid function status of the participants according to their Anti-TPO Ab status (N=100)		
Thyroid Status	**Anti-TPO Antibody Status**	
	Positive	**Negative**
Euthyroid	21(75.0)	63 (87.50)
Hyperthyroid	02 (07.1)	01 (01.4)
Hypothyroid	05 (17.9)	8(11.1)
Total	28(100.0)	72(100.0)
Fisher's exact probability=0.139 (Euthyroid vs Thyroid Dysfunction)		

Inference: Most of the participants in either group reported a Euthyroid status. Only two (7.1%) in the Anti-TPO Ab positive group and one (1.4%) in the negative group had hyperthyroidism. Similarly, only five (17.9%) in the former and eight (11.1%) in the latter were hypothyroid. When the hyper and hypothyroid participants were grouped together, we observed that no significant difference existed between the participants according to their Anti-TPO Ab (p=0.139).

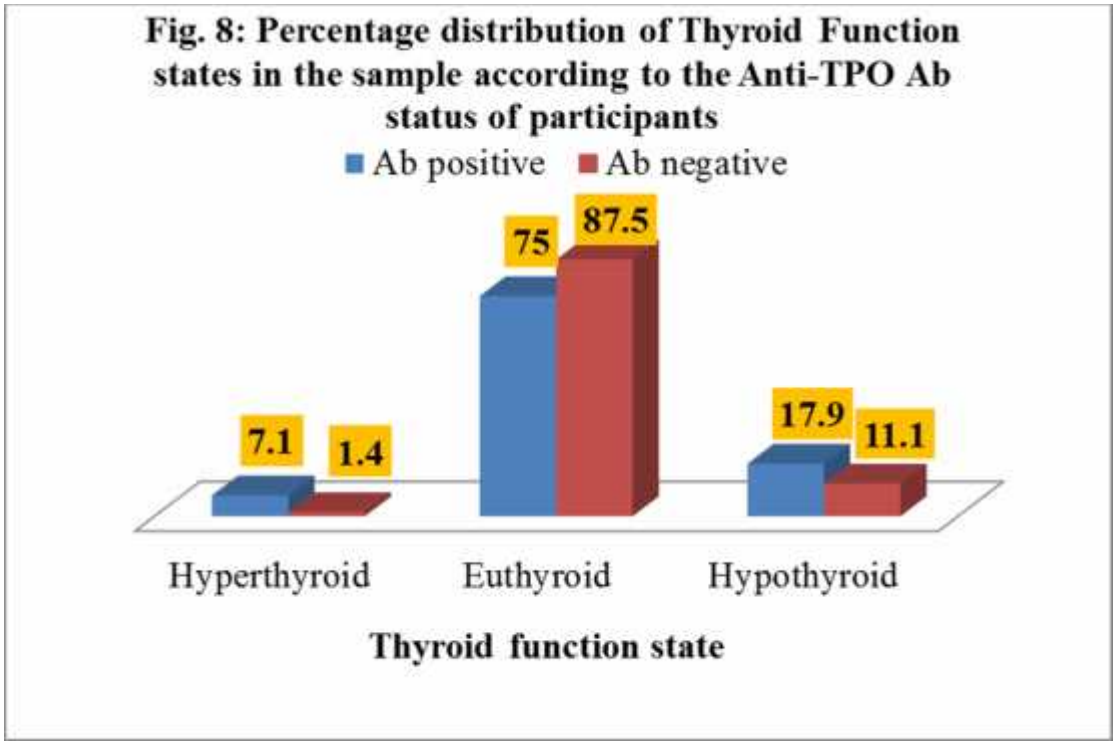
Fig. 8: Percentage distribution of Thyroid Function states in the sample according to the Anti-TPO Ab status of participants
Ab positive
Ab negative
7.1
1.4
75
87.5
17.9
11.1
Hyperthyroid
Euthyroid
Hypothyroid
Thyroid function state

Table 28 : Associated disease conditions according to Anti-TPO Ab status of the study participants (N=100)

Sl. No.	Associated disease	Anti-TPO Antibody Status		P value (Fisher's exact probability)
		Positive (N=28) No. (%)	Negative (N=72) No. (%)	
1	Systemic	0	04 (05.6)	0.574
2	Autoimmune	02 (07.2)	0	0.076
3	Dermatological	02 (07.2)	03 (04.2)	0.617

Inference: Autoimmune disease history was found in two of the 28 patients who tested positive for Anti-TPO Ab. One had duodenitis/gastritis with pernicious anaemia and the other, alopecia areata. None of Anti-TPO Ab negative participants provided a positive history for autoimmune diseases. No significant association could be elicited for history of autoimmune diseases with Anti - TPO Ab status (p=0.076).

Among systemic diseases Diabetes Mellitus was reported in four patients who had tested negative for anti-TPO Antibodies; one of these diabetics also had associated bronchial asthma. None of the Anti-TPO Ab positive participants in the study had any history of systemic disease. The association of systemic diseases with Anti-TPO Ab status was statistically insignificant (p=0.574).

No other dermatological disorder was found associated with either of the Anti-TPO Ab status based groups (p=0.617) in the patients with vitiligo included in the study. Irritant contact dermatitis and subacute eczema was found in one patient each with Anti-TPO antibodies. Among those who tested negative for the antibodies, congenital melanonychia, nevus acromicus and apthous stomatits with xerosis were reported in one patient each.

DISCUSSION

Vitiligo is a common acquired dermatological disorder. Most recent data suggest an autoimmune etiopathogenesis. Over the years there have been various reports of associations between vitiligo and other autoimmune diseases notably thyroid disease and antithyroid antibodies. Therefore, we undertook this study to look into the characteristics of vitiligo patients with thyroid autoimmunity as indicated by their Anti-Thyroid Peroxidase Antibody (Anti-TPO Ab) titres.

In the preceding section the results have been presented under two sub-headings that address the study objectives. The discussion has been undertaken correspondingly.

1. Sample characteristics:

Age and sex of the participants:

A total of 100 patients with vitiligo attending the dermatology OPD at KLE's Dr Prabhakar Kore Hospital and Medical Research Centre were recruited consecutively in this study starting from January, 2012. The age of the participants ranged from 2 to 62 years. The mean age of the study participants was 29.49±15.00 years with a median of 28 years. The mean age of males was 33.88+15.86 years (median = 35 years) while that of females was 26.44±13.70 years (median=24 years) *(Table no. 1).* Majority of the participants were aged 20-29 years; however, males were mostly in their forties and females in their twenties. Similar hospital based studies conducted in Brazil[74] and Turkey[83] which included all age groups have also found the mean age to be 37.14 and 31.30 years respectively. In our study majority of the participants were females (59%). Earlier studies have suggested that vitiligo being an autoimmune disease could be more common in females[5,12,18,19]. Moreover, since

vitiligo causes cosmetic aberrations, it could be generating higher concern among females, leading to an increased attendance of females among vitiligo patients in the Dermatology OPD as compared to males.

Reported age at onset of vitiligo:

Vitiligo may appear at any time shortly after birth to senescence although an onset between 5-30 years of age is most common[3]. The mean participant reported age at onset in our study was 24.80±0.16 years (median: 21.25 years; range: 1.67-63 years) *(Table 2).* Other investigators have also reported that majority of vitiligo cases have an onset in and around 2nd and 3rd decade of life[18,19,31,83]. However, a study in India reported a later onset of the disease, with a mean age of 55 years[84]. These data reinforce that vitiligo is a disease that can occur at any age. The age at onset of vitiligo in the male participants (n=41) was 30.43±15.88 years while it was 20.89±14.38 years in the females (n=59). Thus, in our study, females manifested vitiligo around 10 years earlier than males (Mann-Whitney U=801, p=0.004). This trend was opposite to that noted by Nordlund and Majumdar[31] who in their study found that males had a seven year early onset of vitiligo than females. Other researchers found no significant difference in age at onset between the two genders [14,42].

Duration of vitiligo:

The mean duration of vitiligo in our study participants was 4.64±6.05 (Median=2) years which is close to the finding of another Indian study with 3.7 years[72]. We did not get a statistically significant difference between males and females regarding this *(Table no. 2).*

Family history of vitiligo:

Many studies have also reported a positive family history of vitiligo in up to 31% of the cases [18 ,31,51]. In our study, a positive family history of vitiligo was found in 13% of the participants which is similar to the findings by Hann et al[43] *(Table no. 3)*. A lower familial aggregation in our study could be possibly because it was reported by participants' recall.

History of precipitating factors:

We probed to find out if the participants could recollect any particular factor that precipitated vitiligo in them for the first time. In our study while majority (92%) of them did not relate to the incidence of vitiligo lesions with any precipitating factor, we found history of emotional stress in two percent of participants; three percent reported that the vitiligo lesions in them manifested at sites of trauma. While one percent attributed vitiligo precipitation to physical stress, another reported sun exposure as the triggering factor for vitiligo lesions. One of the females recollected the first appearance of vitiligo during pregnancy *(Table no. 4)*. Emotional stress has been mentioned as a triggering factor in other studies[52], but these data are still limited and there is no established evidence in the literature. In some studies, history of a precipitating factor (physical injury being most common that is Koebner's phenomenon followed by emotional stress, sunburn, systemic illness, pregnancy, and parturition) has been shown to be present in 10-76% of cases, but the high and sometimes concomitant occurrence of these factors as such makes it difficult to assess the exact role played by them in precipitating vitiligo[34].

Disease activity:

Vitiligo disease activity score (VIDA) is a six-point scale for assessing vitiligo activity[85]. Scoring is based on the individual's own opinion of the present disease activity over time. Active vitiligo involves either expansion of existing lesions or appearance of new lesions. Grading is as follows: VIDA Score '+4' - Activity of six weeks or less duration; '+3' - Activity of six weeks to three months; '+2' - Activity of three to six months; '+1' - Activity of six to twelve months; '0' - Stable for one year or more; and '-1' - Stable with spontaneous repigmentation since one year or more. A low VIDA score indicates less activity. Majority of the participants reported relatively low disease activity with a VIDA score of '1' in 55% cases and VIDA score of '0' in 14% cases. Only one patient reported spontaneous repigmentation with a VIDA score of '-1' at the time of repigmentation *(Table no. 5).* We could not find relevant literature that have looked into VIDA scores in vitiligo patients in a contextually relevant setting.

Types of vitiligo:

Among non-segmental vitiligo, vitiligo vulgaris was the commonest (71%) type of vitiligo in our study followed by focal vitiligo (12%), mucosal vitiligo (6%), acral vitiligo (5%) and acrofacial vitiligo (2%). Other investigators also found vitiligo vulgaris to be the commonest type of vitiligo in their case series with 60.5%[61] and 69.5%[51]. In a study done by Song et al[42], the incidence of the generalized type of vitiligo was 50.2% (vitiligo vulgaris: 35.8%; acrofacial vitiligo: 14.4%) while that of localized vitiligo was 49.7 % (segmental vitiligo: 15.4%; mucosal vitiligo: 0.6%). But mucosal vitiligo was reported to be much higher (22.8%) in another Indian study[72]. No cause could be postulated for this difference. In our study, segmental vitiligo was

found in only 4% of the cases. The reported prevalence in literature of segmental vitiligo ranges from 3.5% to 20.5% of all patients with vitiligo[47] *(Table no. 6).*

Site of onset of vitiligo:

With regard to the site of onset, the upper limbs were the most frequently affected in the study by Nunes DH[74]. They were also the most commonly affected site of onset (77.9%) in an Indian study[84]. Handa and Kaur[86] reported the initially developing site of depigmentation to be face, trunk, and legs in decreasing order of frequency. These data suggest that the primary site of involvement are sun-exposed areas. While another study from Kuwait[87] noted majority of their patients beginning depigmentation in lower limbs followed by upper limbs, head and neck, trunk and mucosae in decreasing order. In our study also majority of the subjects i.e. 34% had lower limbs as the site of onset followed by head and neck, and trunk with each 22% and upper limbs in 20%, and genitals 2% in decreasing order *(Table No. 7).*

Current site of involvement:

The most common site of involvement in our study population was lower limb (75%) followed by upper limb (52%), head & neck (42%), trunk (42%), mucosa (17%) and genitals (9%) *(Table No. 7).* These findings are similar to another study where the most commonly affected area was upper limb (63.5%) followed by face (58.8%), lower limb (57.6%) and trunk (49.4%) in their vitiligo patients[74]. SB Cho et al[78] noted the most commonly involved site to be the face (54.6%).

Vitiligo Area Severity Index (VASI)[85]:

The percentage of vitiligo involvement is calculated in terms of hand units. One hand unit (which encompasses the palm plus the volar surface of all digits) is

approximately equivalent to 1% of the total body surface area. The degree of pigmentation is estimated to the nearest of one of the following percentages: 100% - complete depigmentation; 90% - specks of pigment present; 75% - depigmented area exceeds the pigmented area; 50% - pigmented and depigmented areas are equal; 25% - pigmented area exceeds depigmented area; and 10% - only specks of depigmentation present. The VASI for each body region is determined by the product of the area of vitiligo in hand units and the extent of depigmentation within each hand unit measured patch.

Total body VASI = ∑ All body sites [Hand Units] * [Residual depigmentation]

Most of our participants (60%) reported VASI between the range of 1-4.99. This should be the usual presentation of vitiligo cases at the dermatology OPD. It could indicate that patients with a score of '<1' usually do not seek health care. Simultaneously, a higher VASI could reflect negligence for the lesions or rapid progression *(Table no. 8).* We could not find other studies which have assessed VASI on vitiligo patients but the prevalence of body surface area involved (calculated by rule of nines) was seen to vary from 5 to 50% in various studies[74,75].

Clinical variants:

The present study noted trichrome and quadrichrome variant (6% each) in the participants *(Table no. 9).* In another Indian study the trichrome variant was seen in 71.4% of their cases which is much higher than our finding[72].

Clinical characteristics of vitiligo lesion:

Koebner's phenomenon was seen in 5% of our cases. This is same as the Indian study by Handa and Kaur (5%)[86] but this being lower than what has been reported by other investigators ranging from 21% to 57.9% [43,51,52,87]. No possible cause

for a lower detection of Koebner's phenomenon in our cases could be ascertained *(Table No. 10).* Leukotrichia or poliosis was present in 14% of our cases. This has been variously reported in other studies from 11.5% to 43.5% [43,51,86,87]. Halo naevus was found in only one patient in our study while another study has reported in seven of their patients who were all children[85].

Association with other diseases

***i. autoimmune diseases*:**

History of autoimmune disease was found in two cases of vitiligo. One was of duodenitis/gastritis with pernicious anaemia while the other provided a history of alopecia areata *(Table 11).* Alopecia areata has been reported with varying frequencies in vitiligo patients - 0.4% to 6.5% [19,51,59,86]. Other autoimmune diseases mostly reported with vitiligo are pernicious anaemia (1.8% to 2.3%), rheumatoid arthritis (0.38% to 14%) and type 1 diabetes mellitus[88, 89, 90, 91].

ii. dermatological diseases:

Handa and Kaur [86] observed atopic/nummular eczema in 49 and psoriasis in 2 out of their 144 participants. SB Cho et al [79] found that 11.7% of their study participants with vitiligo presented with a combined disease and the most commonly associated disease was atopic dermatitis. In our study one case each of apthous stomatits with xerosis, irritant contact dermatitis, congenital melanonychia, nevus acromicus and subacute eczema were seen in the 100 participants *(Table no. 11).*

iii. systemic diseases:

Out of 100 vitiligo patients in our study, only four had diabetes mellitus type 2. One of them also had a concomitant history of asthma *(Table no. 11).*

2. Prevalence of thyroid autoimmunity in the study sample:

The upper limit for anti-TPO Ab was as per the hospital laboratory reference range. We defined an anti-TPO Antibody serum titre of ≥35 IU/ml as *'anti-TPO Ab positive'* and evaluated the same in these 100 participants. This was used a proxy indicator for thyroid autoimmunity in the study participants. Twenty eight of the 100 participants had a positive titre. Thus, the sample prevalence of anti-TPO antibody positivity (thyroid autoimmunity) was estimated at 28% (95% CI for population prevalence: 19.1%-37.0%). Previous researchers have reported prevalence of anti-TPO antibody positivity in vitiligo patients from around 11% to as much as 50% *(Table iv).*

Table No. iv: Comparison of thyroid autoantibody profile in various studies

Sl. No.	Studies	Reported prevalence (%) of Anti-TPO Ab in vitiligo patients
1	Cunliffe et al (1968)[5]	11.6
2	Schalleruter et al (1994)[51]	10.7
3	Hegedus et al (1994)[60]	25.2
4	Mandry et al (1996)[68]	50
5	Dave S. et al (2003)[72]	17.1
6	Daneshpazhooh M. et al (2006)[75]	18.1
7	Altaf H. et al (2011)[73]	11
8	Our study in discussion (2013)	28

The estimated prevalence in our study lies within the reported range in the available literature. However, we should bear in mind that demographic characteristics and health profile of the participants in studies discussed play determining roles in the prevalence estimates. Care seeking behaviour of the OPD attendees and willingness to participate in the study also affect the estimation of prevalence. Moreover, hospital recruited samples may not be true representative of the general population and could under-estimate disease prevalence if the care seeking behaviour is poor. Nevertheless, our study site being a popular health care set-up in the region, we believe that the sampled participants could provide an approximate population estimate of the catchment area for diseases like vitiligo where the patient usually seeks medical advice. However, this again could be an under estimation in the presence of other healthcare providers in the region.

3. Characteristics of participants in association with thyroid autoimmunity:

Profile of participants with a serum concentration of anti-TPO Ab >35 IU/ml *('anti-TPO Ab positive')* was compared with those with a concentration of 0-35 IU/ml *('anti-TPO Ab negative').* Thus, of the 100 participants, 28 were in the former and the remaining 72 in latter category.

Age and sex distribution of participants:

The mean age of the Anti-TPO Antibody positive participants was 34.5 years as compared to 27.5 years in the antibody negative participants. The standard deviations in both showed a skewing of the age distribution. Non-parametric comparisons (Mann Whitney U test) found a significant difference (p=0.037) *(Table 13).* The distribution of the participants when grouped into decadal age groups showed that the participants in the anti-TPO negative group were mostly their second

decade of life while representation consistently decreased in the subsequent decadal age bands. Participants in the anti-TPO Ab positive group were mostly in their third decade and beyond *(Table 14).* Both Mandry et al[68] and Nunes DH[74] have reported that their patients with autoantibodies tended to have a higher mean age when compared to patients without autoantibodies which is in accordance to our findings. In the currently discussed study, the proportion of females in the Anti-TPO Ab positive group, was higher (71.4%) than in the Anti-TPO Ab negative group, (54.2%), the female:male ratio in the two groups being 5:2 and ~5:4 respectively *(Table 15)*. However, sex predilection for females in either group was not statistically significant (p=0.115). Both Altaf H et al[73] and Nunes DH[74] observed a significantly higher incidence of thyroid dysfunction as well as anti-TPO Ab positivity in females in their respective studies. Maryam D et al[75] reinforced this finding as they noted, anti-TPO Ab to be significantly more common in females patients especially in young women in the age range of 18 to 35 years, compared with control group.

Reported age at onset of vitiligo in participants and its correlation with their gender:

The mean age at onset of vitiligo (in years) in anti – TPO Ab positives (24.59) was not much different from that (24.88) in anti-TPO Ab negatives. It was not statistically significant (p=0.982) *(Table 16).* Earlier investigators have reported that their patients with autoantibodies tended to have a later onset of disease when compared to patients without autoantibodies[68,74]. However recently, Altaf H et al[73] found that a significant number (86%) of patients with anti-TPO antibody positivity had early onset vitiligo. Dave and colleagues also found earlier age at onset to be associated with thyroid abnormality but without statistical significance[72].

Duration of vitiligo:

Those participants testing positive for anti-TPO antibodies reported a longer duration of vitiligo as compared to those who tested negative. The mean duration of vitiligo in the former group was around five years as compared to 4.4 years in the latter *(Table 16).* However, this was not statistically significant (p=0.7). Betterle[54] showed a significant relationship between anti-TPO Ab with long lasting vitiligo in their patients.

Family history of vitiligo in participants:

History of vitiligo in the family was reported by 10.7% of our anti-TPO positive patients while it was 13.9% in anti-TPO negative patients. We enquired if history of vitiligo in the family was more commonly elicited in either of the two participant groups but found no such association *(Table 17).* Family history of vitiligo was seen in 5% of the cases with thyroid abnormality in the study by Dave S et al[72].

History of precipitating (trigger) factors:

A statistically significant history of coincident triggering factor with vitiligo was elicited commonly in those with positive Anti-TPO Ab status as compared to those with Anti-TPO Ab negative status *(Table 18).* A suitable reason for the association of trigger factors being commoner in those participants with Anti - TPO Ab positivity than those without could not be ascertained and probably a larger study over a longer duration of time measuring association of thyroid autoimmunity with individual precipitating factors in vitiligo would be able to determine whether or not presence of trigger factors can be considered to predispose to associated thyroid autoimmunity in vitiligo patients.

Disease activity in the participants:

A low VIDA score indicates less activity. In our study, (19.4%) of the Anti-TPO Ab negative participants had a VIDA score of '0' or '-1' as compared to only 3.6% of those found positive. All but one (96.4%) of the Anti-TPO Ab positive participants reported disease activity with a VIDA Score '≥1' indicating that anti-TPO Ab positivity could be associated with progressive vitiligo disease activity *(Table 19).* This could also suggest that majority of our cases could be of type A or autoimmune vitiligo which is known to progress continuously with alternate periods of remissions and exacerbations[48].

Type of vitiligo and clinical variants:

Vitiligo vulgaris was the commonest type of vitiligo in our study irrespective of anti-TPO Ab status *(Table 20).* In an Indian study by Dave and colleagues, mucosal vitiligo was found to be significantly associated with thyroid autoimmunity[72] but in our study no significant difference was found in the representation of segmental and non-segmental vitiligo in the two groups. None of the 50 patients with segmental vitiligo showed any thyroid dysfunction (P = 0.047) in the study by SB Cho et al [79].

Site of onset of vitiligo:

With regard to the site of onset, while an equal number of participants in the Anti-TPO Ab positive group reported an onset of vitiligo in head and neck, trunk, upper limb and lower limb (25% each), none reported genitals as a site of onset. In the Anti-TPO Ab negative group, majority had onset of vitiligo in the lower limb (37.5%) followed by head and neck and trunk regions (20.8% each) *(Table 21)*. We

did not find significant association of site of onset with anti-TPO Ab status which is in accordance with other studies that have evaluated the same[79].

***Vitiligo Area Severity Index (VASI)*[85]:**

The mean VASI percentages in the anti-TPO positive group was 2.40 and anti-TPO negative was 3.12 but it did not have a significant difference (p=0.376). We could not find other studies which have compared VASI with anti-TPO Ab *(Table 22)*. But a significant association of greater body surface area involved (calculated by rule of nines) with positive thyroid antibodies reported by Nunes DH et al[74].

Clinical variants:

While nearly 10% of the participants with Anti-TPO Ab positivity had the trichrome variant of vitiligo, the proportion was just 4.2% for their Anti-TPO Ab negative counter-parts. Similarly around 7% of the participants with Anti-TPO Ab positivity had the quadrichrome variant of vitiligo, the proportion was 5.2 % for their Anti-TPO Ab negative counter-parts. Majority (82.1% and 90.3% participants in these respective groups) could not be identified with any particular variant *(Table 23)*.

Clinical characteristics of vitiligo lesion:

Even as we obtained a p value of 0.132, we note that koebnerization was more frequently reported in Anti - TPO Ab positive participants (10.7%) as compared to the Anti - TPO Ab negative ones (2.8%). Leukotrichia was equally frequent in both the groups (around 86% in both) *(Table 24)*. Halo nevus was present in only one patient who was anti-TPO Ab negative. K. Ezzedine et al noted the presence of halo naevi, circulating antithyroid antibodies and leukotrichia more often in patients with mixed vitiligo (65.4%, 23.1% and 92.0%, respectively) than those with segmental vitiligo (12.0%, 5.9% and 36.0%, respectively). Thus, they inferred halo naevi

association and leukotrichia at first consultation in segmental vitiligo as risk factors for the progression of segmental vitiligo to mixed vitiligo[92].

***Association of anti-TPO Ab with thyroid status*:**

The serum TSH levels in either group showed a skewed distribution with no significant difference (p=0.514). However, the mean TSH level was almost five times higher in Anti - TPO Ab positive participants than the Anti - TPO Ab negative subjects *(Table 25).* We explored if serum TSH levels had a correlation with the Anti-TPO Ab antibody titre. A correlation coefficient of 0.052 disproved this and highlighted that these are probably mutually unrelated (p=0.609) *(Table 26).* Previously Adel A. Imam et al[78] have noted a positive significant correlation between serum anti-TPO Ab and serum TSH. In the present study, most of the participants in either categories of the reported an euthyroid status. Only two (7.1%) of the Anti-TPO Ab positive participants and one (1.4%) of the negative particpants had hyperthyroidism. Similarly, only five (17.9%) in the former and eight (11.1%) in the latter were hypothyroid *(Table 27).* We observed that no significant association was present between the Anti-TPO antibody status and the thyroid function status (euthyroid versus thyroid dysfunction) of the study participants (p=0.126). An Indian study by Dave and colleagues[72] reported that biochemical abnormality (predominantly hyperthyroidism) was found in 40% of the cases as against 6.7% of the controls ($p<0.05$).

Association with other diseases:

We did not find any significant association of coexistent autoimmune, dermatological or systemic diseases with anti-TPO Ab status of the study participants.

CONCLUSION

Results obtained from various studies conducted upon patients with vitiligo have shown it to be intricately associated with thyroid antibodies. In this background, an assessment of Anti – TPO Ab in relation to vitiligo was made on 100 patients. In our study, the attendance of females (59%) was considerably higher than males (41%). Females manifested vitiligo around 10 years earlier than males and this difference was significant. The prevalence of anti-TPO antibodies (serum titre $\geq$35 IU/ml), taken as a proxy for thyroid autoimmunity, was found to be 28%. Previous studies by Mandry et al detected a much higher prevalence (50%) of anti-TPO Ab in vitiligo cases; similar number was seen in the study by Kurtev and Dourmishev on children and adolescents with vitiligo but recent Indian studies by Dave S et al and Altaf et al found anti-TPO Ab in 17.1% and 11% of their vitiligo cases. Our study is most similar to findings of an older study by Hegedus et al who found anti-TPO Ab in 25.2% of vitiligo patients. We found a significant relationship of anti-TPO Ab with a higher mean age of the study sample. Majority of the participants were in their third decade and beyond which is in accordance with the study by Mandry et al. Even though not statistically significant we noted cases with longer duration of vitiligo cases were more commonly associated anti-TPO Ab positivity which goes with the finding by Betterle et al who also observed significant association of thyroid autoantibodies in long standing vitiligo. A unique finding of our study was significant association of anti-TPO Ab with presence of coincident precipitating 'trigger' factor which has not been observed by previous researchers. Emotional stress has been mentioned as a triggering factor in other studies by Huggins RH and Sehgal VN, but limited data is available regarding association of antibodies with trigger factors for vitiligo and there is no established evidence in the literature.

In their study Mandry et al noted that despite the high percentage of autoantibody positivity (75%) in patients with vitiligo, the presence of overt autoimmune/endocrine disease was noted in only 14% of the cases implying the high incidence of latent disease which would probably manifest only during follow up. Similarly none of our patients had known thyroid dysfunction at the time of investigation for vitiligo. This is in conformity with findings of other studies, that vitiligo often precedes thyroid dysfunction. Hence screening vitiligo patients for thyroid autoimmunity so as to diagnose and initiate treatment for any co-morbidity with the above test seems to be relevant as this antibody is sensitive and specific marker of autoimmune thyroid disorders. This is especially recommended in older patients, more so in female patients, and those giving history of a precipitating factor and or history of longer duration. Perhaps, studies on a larger number of vitiligo patients with control group from general population who can be followed up for a considerable time, would further strengthen these associations and also bring forth if any other specific clinical parameters of vitiligo could raise suspicion for the associated of thyroid autoimmunity.

SUMMARY

- This was a cross-sectional study was carried out from January 2012 to December 2012.
- The source of data were vitiligo patients attending the Dermatology OPD, at KLES Dr. Prabhakar Kore Hospital and MRC, Belgaum.
- The objectives of the study was to evaluate the prevalence of thyroid autoimmunity in 100 vitiligo cases by measuring anti-TPO Ab levels by quantitative chemiluminescent immunoassay in the serum samples collected from the each of them with due consent.
- The proportion of females was higher (59%) than males (41%).
- The age of the participants broadly ranged from 2 to 62 years. The median age of males was 35 years while that of females was 24 years. Majority of the participants were aged 20-29 years irrespective of gender. However, males were mostly in their forties and females in their twenties.
- The median participant reported age at onset was 21.25 years; range: 1.67-63 years. Females manifested vitiligo around 10 years earlier than males and this difference was statistically significant.
- The median duration of vitiligo of the participants was 2 years.
- A positive family history of vitiligo was found in 13% of the participants, history of precipitating factors was present in 8 %, history of associated autoimmune disease in 2%, associated dermatological diseases in 5% and associated systemic illness in 4%.
- Segmental vitiligo was found in only 4% of the cases. While among non-segmental vitiligo, vitiligo vulgaris was the commonest (71%) type of vitiligo

in our study followed by focal vitiligo (12%), mucosal vitiligo (6%), acral vitiligo (5%) and acrofacial vitiligo (2%).

- Majority of the subjects (34%) had lower limbs as the site of onset of vitiligo followed by head and neck, and trunk with each 22% and upper limbs (20%), and genitals (2%). While the most common affected site in our study population was also lower limb (75%) followed by upper limb (52%), head & neck (42%), trunk (42%), mucosa (17%) and genitals (9%).
- Majority of the participants reported relatively low disease activity with a VIDA score of '1' in majority (55%) cases.
- Most of our participants (60%) reported VASI between the range of 1-4.99.
- We noted trichrome and quadrichrome variant of vitiligo in 6% (each), Koebner's phenomenon in 5% and leukotrichia in 14% of our cases. One patient had halo naevi.
- 28% of the participants had Anti – TPO antibodies in the study sample.
- In the age distribution, a significant difference was found between the anti-TPO Ab positive versus negative groups with majority of the participants being in their third decade and beyond.
- The mean participant reported age at onset of vitiligo, mean duration of vitiligo or site of onset, none were significantly associated with anti-TPO Ab.
- Positive family history of vitiligo was not found significant in association with anti-TPO Ab neither was the association history of other autoimmune diseases, dermatological diseases or systemic diseases in participants.
- A history of coincident triggering factor with vitiligo was elicited more commonly with those with positive Anti - TPO Ab status as compared to

those with Anti - TPO Ab negative status. The difference was significant with a p value was 0.037.

- All but one (96.4%) of the Anti-TPO Ab positive participants reported disease activity with a VIDA Score '≥1' indicating that anti-TPO Ab positivity could be associated with progressive vitiligo disease activity.
- No significant difference was found in the representation of segmental and non-segmental vitiligo or its subtypes in either anti-TPO Ab positive or negative groups.
- The VASI percentages in the groups did not have a significant difference (p=0.376).
- Association with anti-TPO Ab with clinical variants such as trichrome or quadrichrome vitiligo, halo nevi, Koebner's phenomenon, leukotrichia, none were found significant.
- The serum TSH levels in either group showed a skewed distribution with no significant difference. The mean TSH level was almost five times higher in Anti - TPO Ab positive participants than the Anti - TPO Ab negative subjects but there was no correlation serum TSH levels and Anti-TPO Ab antibody titre. 7.1% in the Anti-TPO Ab positive group and 1.4% in the negative group had hyperthyroidism. Similarly, 17.9% in the former and 11.1% in the latter were hypothyroid. We observed that no significant association was present between the Anti-TPO antibody status and the thyroid function status of the study participants (p=0.126).

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ANNEXURE I

INFORMED CONSENT FORM

I.D.NO. _____

One year cross sectional study of evaluation of association between Vitiligo and Thyroid Autoimmunity.

The study is conducted by Post graduate student in M.D Dermatology Dept of Dermatology, Venereology, Leprosy, JNMC, KLE University, Belgaum.

Respected Sir/Madam, we invite you to participate in our study as, you are eligible for the same. During the study you will be asked some questions in detail regarding your present complaints.

Purpose of the study:

The purpose of this study is to find out the correlation of thyroid dysfunction in vitiligo. You are being asked to participate in this research because you have been diagnosed to have vitiligo. All patients attending the outpatient department, who are diagnosed to have this disease, will be requested to participate in this study during the period of one year.

Procedure and treatment:

Should you choose to participate, you will be asked to give a detailed history of your disease, undergo a physical examination, and consent to a few routine blood and urine investigations. In addition to this, you will agree to undertake TSH & ANTI-TPO test and if required other relevant investigations.

Risks and benefits:

You may undergo some amount of discomfort during the process of investigations, which may include slight pain and bleeding. However all necessary steps and precautions will be taken to ensure your safety. The result of you taking part in this research would help health care providers towards a better understanding of this disease, and thus we will be able to provide improved patient care

Alternatives:

If you decide not to participate in this study, you will still be receiving the usual standard care for your disease.

Privacy and confidentiality:

Your privacy will be respected and all information collected about you during the course of this study will be kept confidential. Your identity will remain undisclosed.

Relations with the Institutional policy:

The J N Medical College will provide, within the limitations of the laws of the State of Karnataka, facilities and medical attention to patients who suffer injuries as a result of participating in this project. In the event if you suffer any physical injury as the result of your participation in this study, you may contact Dr._____ on mobile no_____.

Financial incentives:

You shall not be receiving any payment or any financial incentives for participating in this study.

Authorization to publish results:

The results of this study may be published for scientific purpose or presented to a scientific group. Your identity, however, will be maintained confidential at all times.

Voluntary participation:

Your participation in this study is voluntary. Your decision whether or not to participate will neither affect the care of your current disease, nor your future relations with the doctor or the hospital. In case you need further information regarding your rights as a study participant, you may please contact If you have any questions about rights as a research participant you can contact Dr. P. V. Patil, Chairman, J. N. M. C Institutional Ethics Committee on human subjects' research on 0831-2471350.

Statement of Consent:

I.D.NO:_____

I Mr/Ms/Mr_____

volunteer and consent to participate in this study. I have read the consent document or it has been read to me in my vernacular language. I accept to participate in the study. All the information regarding this study is provided to me and I have understood the same. I have been given the opportunity to ask questions and obtain appropriate answers.

Participant's name:

Signature or left thumb print of participant:

[If the participants are Minors (under 18), the parents sign the form, rather than the participants]

Witness name:

Signature of witness & Date :

Signature of the investigator & Date :

ANNEXURE II

PROFORMA

One year cross sectional study of evaluation of association between

Vitiligo and Thyroid Autoimmunity

Case No. ☐☐ **OP/IP No.** ☐☐☐☐☐☐☐

Name:

First name Middle name Last name

Age: ☐☐

Sex: 1. Male ☐

2. Female

Occupation: _____

Income: (In Rs.):

Address with phone number:

Presenting complaints and duration:

History of present illness:

1. Age of onset:

2. a. Onset: 1.Sudden 2.Gradual

b. Course: 1.Unstable 2.Stable

VIDA :

3. Precipitating factors if any:

1. Trauma
2. Chemical

3. Stress
4. Sun exposure
5. Others

4. Site of onset of lesion:

5. Current site of lesion:

Face : 1.Present 2.Absent

Oral cavity : 1.Present 2.Absent

Neck : 1.Present 2.Absent

Back : 1.Present 2.Absent

Trunk : 1.Present 2.Absent

Upper Extremity : 1.Present 2.Absent

Lower Extremity : 1.Present 2.Absent

Palms : 1.Present 2.Absent

Soles : 1.Present 2.Absent

External genitalia: 1.Present 2.Absent

VASI :

Any associated factors:

1. Itching
2. Pain
3. Burning
4. Asymptomatic

Visual abnormalities : 1.Present 2.Absent

Hearing abnormalities : 1.Present 2.Absent

History suggestive of Thyroid Disorder:

1. Present
2. Absent
3. If yes, Duration_____months/years

History suggestive of Asthma/Atopy:

1. Present
2. Absent
3. If yes, Duration_____months/years

History suggestive of associated systemic illness:

1. Present
2. Absent
3. If yes, Duration _____months/years

History suggestive of any other autoimmune conditions:

1. Present
2. Absent
3. If yes, Duration_____months/years

History of any other dermatological disorders:

1. Present
2. Absent
3. If yes, Duration_____months/years

Past History:

Family History of vitiligo:

Treatment History

Phototherapy 1-On treatment

2-Not on treatment

Chemotherapy 1-On treatment

2-Not on treatment

Alternative treatment if any :

Personal History:

Diet: 1.Mixed 2.Veg

Appetite 1.Normal 2.Poor

Bowel/ Bladder : 1. Normal 2.Altered

Sleep: 1. Normal 2.Altered

Alcohol : 1. Yes 2.No

Smoking: 1. Yes 2.No

General Physical Examination:

Built: 1. Poor 2.Moderate 3.Good

Pulse / min:

BP (mm/hg):

Temperature: ____ ^{0}F

Weight (Kg)

Pallor : 1.Present 2.Absent

Icterus : 1.Present 2.Absent

Cyanosis: 1.Present 2.Absent

Clubbing : 1.Present 2.Absent

Lymph nodes: 1. Palpable 2. Non palpable

Edema: 1.Pitting 2. Non Pitting 3. Absent

Mucocutaneous Examination:

Type of lesion:

Macules: 1.Present 2.Absent

Patches: 1.Present 2.Absent

Distribution:

Nordlund

A. Localised

B. Generalised : 1. Symmetrical 2. Asymmetrical

C. Universalis

Koga

A.Vitiligo Non-Segmentalis

B. Vitiligo Segmentalis

Special Type of lesion:

1. Trichrome
2. Quadrichrome
3. Pentachrome
4. Vitiligo ponctue
5. Vitiligo with raised borders

Associated features:

Atrophy	1.Present 2.Absent
Scaling	1.Present 2.Absent
Telengectasia	1.Present 2.Absent
Kobener's Phenomenon	1.Present 2.Absent
Leukotrichia	1.Present 2.Absent
Blisters	1.Present 2.Absent
Erosion	1.Present 2.Absent
Peeling of skin	1.Present 2.Absent
Erythema	1.Present 2.Absent

Mucosal Examination:

Genital lesion: 1.Present 2.Absent

Oral lesion: 1.Present 2.Absent

Hair Changes: 1.Present 2.Absent

Nail Changes: 1.Present 2.Absent

Systemic Examination:

Cardiovascular system: 1. Normal 2. Abnormal

Respiratory system: 1. Normal 2. Abnormal

Per abdomen: 1. Normal 2. Abnormal

Central nervous system: 1. Normal 2. Abnormal

Investigations :

Anti TPO

TSH

Diagnosis:

Signature:

Guide's Signature:

Date:

ANNEXURE: III

PHOTOGRAPHS

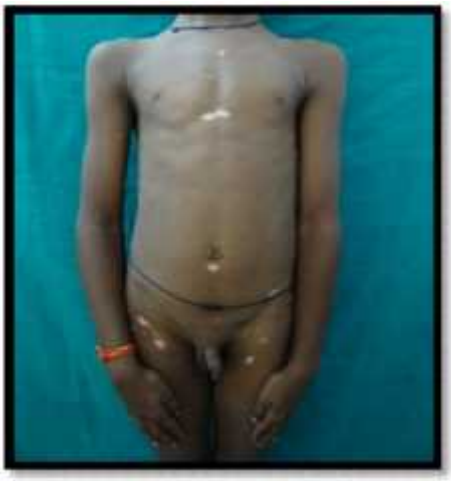

PHOTO 1: VITILIGO VULGARIS

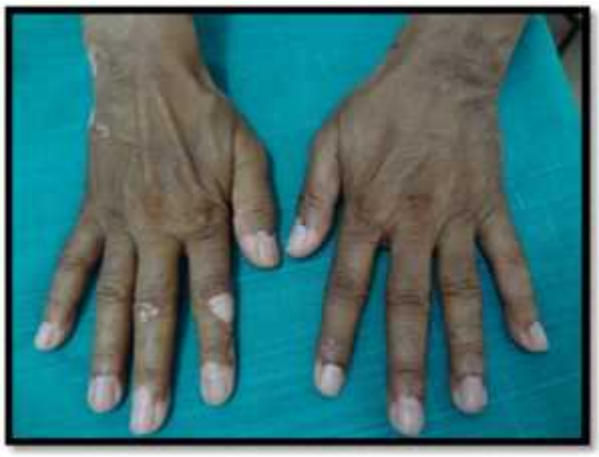

PHOTO 2: ACRAL VITILIGO

PHOTO 3: SEGMENTAL VITILIGO WITH LEUCOTRICHIA

PHOTO 4: MUCOSAL VITILIGO

PHOTO 5: TRICHROME VITILIGO

PHOTO 6: HALO NAEVI

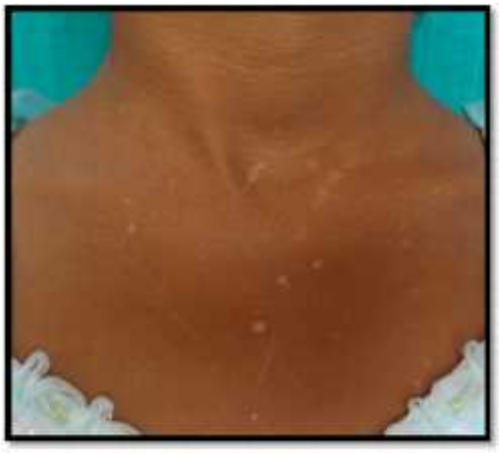

PHOTO 7: KOEBNER'S PHENOMENON IN VITILIGO

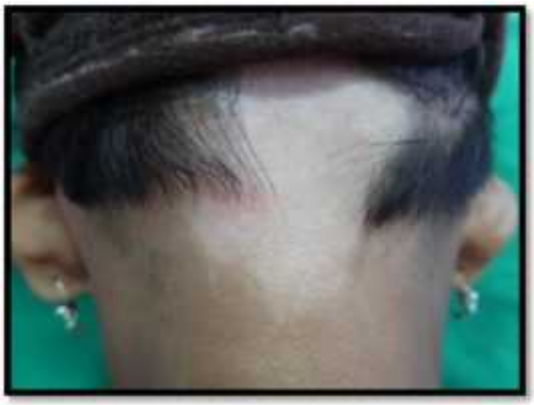

PHOTO 8: ALOPECIA AREATA CONCOMITANT WITH VITILIGO

ANNEXURE: IV

MASTER CHART

Introduction

Aim & Objectives

Review of Literature

Methodology

Results

Discussion

Conclusion

Summary

Bibliography

Annexure-I

Consent Form

Annexure-II

Proforma

Annexure-III

Photographs

Annexure-IV

Master Chart

Annexure VII

KEY TO USE MASTER CHART

- *ES : Emotional Stress*
- *PS : Physical Stress*
- *Q : Quadrichrome*
- *T : Trichrome*
- *Ap : Apthous Stomatitis*
- *AA: Alopecia areata*
- *Sc : Subacute eczema*
- *Se : Sun exposure*
- *Tr : Trauma*
- *CM : Congenital melanonychia*
- *X : Xerosis*
- *Du : Duodenitis*
- *PA : Pernicious anaemia*
- *Pr : Pregnancy*
- *NA : Naevus achromicus*
- *ICD : Irritant Contact Dermatitis*
- *DM : Diabetes Mellitus*
- *As : Asthma*
- *N : None*
- *P : Present*
- *Ne : Negative*
- *Po : Positive*
- *Yrs : Years*
- *Mn : Months*
- *LL : Lower limb*
- *UL : Upper limb*