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Correlation of the study of autoimmune diseases and skin disorder like Vitiligo and different Thyroid disorders. A Prospective Randomized controlled study done at North-eastern region.

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Abstract
Background and aims: *The precise cause of vitiligo is complex but some evidences are always suggesting that it is caused by a combination of autoimmune, genetic and environmental factors. Over half of the people with vitiligo have acquired some loss of pigment cells before the age of 20 years. The prevalence of the disease is between 1-2% in general population. Therefore, the present study is taken up to evaluate the prevalence of autoimmune diseases in vitiligo patients. This also aims to determine which type of thyroid disease and disorders of thyroid autoantibody has strong possible correlations in etiopathogenesis and clinical spectrum of vitiligo, as this devastating depigmentary disease has become one of the most common skin disorders attending Dermatology OPD in the North-Eastern region of India. autoimmune theory of pathogenesis of vitiligo (Shyria D et al, 2003). In vitiligo, recent findings shows increased prevalence of autoantibodies which includes thyroid peroxidase antibody (anti-TPO) and thyroid globulin antibody (anti-TGA).* ***Methods:*** *The study was conducted in the Department of Biochemistry in collaboration with the Department of Dermatology, from July 2018 to August, 2109. It is a prospective, randomized and controlled study in which 90 vitiligo patients attending the Dermatology OPD and vitiligo clinic are enrolled in the study. Thirty five apparently healthy, age and sex matched individuals are selected to serve as control. Serum total Triiodothyronine (tT3) total tetraiodothyronine (t4) or thyroxine and total stimulating hormone was estimated by Immuno-enzymometric assay using commercially available kit manufactured by Monobind Inc.Lake forest, CA 92630,USA . Datas are entered and computed with SPSS version 15 software and well known statistical test like Chi-square, students 't' test , etc. are used wherever found applicable and necessary.* ***Results:*** *The highest vitiligo cases were observed from Imphal-West district of Manipur with 33 (36.67%) patients followed by Imphal- East 25 (27.78%) cases, and Churachandpur 21 (23.33%) cases, which are situated abit farther away from RIMS Hospital. Religion wise, Hindus contributed maximum number of patients (56.67%). The difference in prevalence rate among different religion is statistically significant with P=.00 and chi-square value 12.11. The minimum age of onset of vitiligo observed in the study is 8 years, proving early onset of the disease in children and adolescents age ranged from 8-62 years in vitiligo group with a mean age of 29.82±14.44 years. The disease is more prevalent in female (70%) as compared to male (30%). Distribution of subtypes of vitiligo with relation to genetic predisposition is statistically significant (P<0.05) with chi-square value (5.21) except for mucosal and universalis subtypes, indicative of a multifactorial causes of vitiligo. This study shows an association of vitiligo with autoimmune thyroid disease along with clinical thyroid diseases. Clinical hypo- thyroidism (37.78%) is predominant over clinical hyperthyroidism (10%), which is statistically significant (P<0.O5). Females (35.56%) are having clinical thyroid dysfunction more than males (23.33%).* ***Conclusions:*** *There is increased incidence of autoimmune thyroid diseases among vitiligo patients. Various thyroid antibodies were detected in thyroid disorders, suggesting that these thyroid antibodies could act as sensitive markers for detection of early and subclinical autoimmune disorders of thyroid gland including Graves' disease and Hashimoto's thyroiditis. The reason being vitiligo usually precedes the onset of thyroid dysfunction. Therefore, these thyroid autoantibodies specially anti-TPO, anti-TG should be included along with thyroid parameters TSH, T_3,T_4 in routine investigation of vitiligo, as association of vitiligo with autoimmune thyroid disease is very much common in the North - Eastern region of India.*

Keywords: Vitiligo, Autoimmune diseases, Thyroid disorders, Anti-TPO, Anti-TG.

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I. Introduction

To date, the pathogenesis of vitiligo is unknown, although there are many theories about its cause including self-destructive, biochemical, neural, autoimmune, and genetic hypothesis. It is the single most important non-neo plastic disease that involves both the immune system and melanocytes which are subsequently destroyed and the affected area turns pale and becomes white. The disease have a distinctive distribution of depigmentary skin patches on the dorsum of the extremities before their 20s. The patches may grow or remain constant in size. Patches often occur symmetrically across both sides on the body. Occasionally small areas may repigment as they are recolonised by melanocytes. The location of vitiligo affected skin changes over time, with some patches repigmenting and affecting other areas. In some cases, mild trauma to an area of skin seems to cause new patches, around the ankles as may caused by friction with shoes or sneakers (Kwinter J et al). Although many theories have been put forward for the etiopathogenesis of vitiligo, the role of immunity claims to be the most popular one. Again, the detection of various antibodies – anti-parietal cells, anti-thyroid, anti- adrenal, anti-smooth muscle cells and many other autoantibodies have further strengthened the autoimmune theory of pathogenesis of vitiligo (Shyria D et al, 2003). In vitiligo, recent findings shows increased prevalence of autoantibodies which includes thyroid peroxidase antibody (anti-TPO) and thyroid globulin antibody (anti-TG). Although many theories have been put forward for the etiopathogenesis of vitiligo, the role of immunity claims to be the most popular one. Again, the detection of various antibodies – anti-parietal cells, anti-thyroid, anti- adrenal, anti-smooth muscle cells and many other autoantibodies have further strengthened the autoimmune theory of pathogenesis of vitiligo (Shyria D et al, 2003). In vitiligo, recent findings shows increased prevalence of autoantibodies which includes thyroid peroxidase antibody (anti-TPO) and thyroid globulin antibody. As stated by Burtis CA et al (1999), thyroid hormones are indispensable for growth and development of body organs and essential for sexual maturation. They control carbohydrate and lipoprotein metabolism, have catabolic effect on adipose tissue as well as muscle. These hormones also have chronotropic/ inotropic effects on heart and calorigenic effects on many other tissues. According to the study of Maryam D et al (2007) thyroid autoantibodies i.e, anti-TPO and anti-TG were assessed among vitiligo patients using enzyme link immunosorbant assay (ELISA). The anti-TPO was shown to be significantly more common in young women with vitiligo as compared to control group. Their study also included evaluation of free T3, free T4 and TSH levels (kits for free T3 and T4 : monobind ; TSH : immunotech). There was no significant difference in the levels of free T3, free T4 and TSH in vitiligo patients as compared to control group. As this antibody is a sensitive tool for detection of autoimmune thyroid disorders, including Graves' disease and Hashimoto thyroiditis, considering the fact that vitiligo usually precedes the onset of thyroid dysfunction. This periodic follow up of vitiligo patients for detecting thyroid diseases is further emphasized, especially in young women who show increased level of anti-TPO.

Occasionally small areas may repigment as they are recolonised by melanocytes. The location of vitiligo affected skin changes over time, with some patches repigmenting and affecting other areas. In some cases, mild trauma to an area of skin seems to cause new patches, around the ankles as may caused by friction with shoes or sneakers (Kwinter J et al, 2007).

The association of vitiligo with autoimmune disorders of other organs like pernicious anaemia, thyroid disease, insulin dependent diabetes mellitus (IDDM) and Addison's disease are well evident. Danesh Pazhooh M et al (2007) stated that vitiligo is an acquired depigmenting disorder due to destruction of melanocytes. Although many theories have been put forward for the etiopathogenesis of vitiligo, the role of immunity claims to be the most popular one. Again, the detection of various antibodies – anti-parietal cells, anti-thyroid, anti-adrenal, anti-smooth muscle cells and many other autoantibodies have further strengthened the autoimmune theory of pathogenesis of vitiligo (Shyria D et al, 2003). In vitiligo, recent findings shows increased prevalence of autoantibodies which includes thyroid peroxidase antibody (anti-TPO) and thyroid globulin antibody (anti-TG). The exact mechanism of action of thyroid hormones on cutaneous receptors is still unclear. However T3 and T4 appears to play a vital and essential role in the formation and growth of hair. Thyroid hormones stimulate epidermal functions which include oxygen consumption, protein synthesis, mitosis and skin layer thickening but dermal effects of these hormones still remains poorly understood. In hypothyroidism the manifestation of skin changes are thickening, hyperkeratosis, diffuse loss of scalp hair and atrophy of nails. It had been evident that increased thyroid hormones in blood always resulted with thin epidermis, soft and fine scalp hair and often accompanied by a diffuse non scaring alopecia (Julia A et al, 2003). Betterly

C et al (1987) pointed out thyroid autoantibodies as a good marker for symptomless thyroiditis. In their study of 3737 subjects without clinically thyroid disorders, they could detect autoantibodies in 7% among normal population, 9% in patients with various non-autoimmune diseases and 11-16% among subjects who either had or were at risk for autoimmune disorders, which include patients with IDDM, vitiligo, alopecia acreata, idiopathic hypoparathyroidism, Addison's disease and those first degree relatives of IDDM, the risk group. Vitiligo can thus be a manifestation of multisystem diseases, therefore clinician should never disregard the disease as merely a cosmetic problem. Screening for autoantibodies is very important, especially if the disease had been long standing . Early detection of clinical or subclinical dysfunction due to impaired target organs need proper treatment. If immunological abnormalities are detected, it is theorytically rational to treat the patients with steroids and other cytotoxic drugs or by plasmapheresis. If the patients sign and symptoms improved with thyroxine treatment, immune suppressive therapy was not deemed necessary or otherwise the complications become more prominent, systematic and debilitating, immune suppressive therapy should be initiated and continued (Nishida W et al, 1990). Therefore, the present study is taken up to evaluate the prevalence of autoimmune diseases in vitiligo patients. This also aims to determine which type of thyroid disease and disorders of thyroid autoantibody has strong possible correlations in etiopathogenesis and clinical spectrum of vitiligo, as this devastating depigmentary disease has become one of the most common skin disorders attending Dermatology OPD in the North-Eastern region of India.

II. Materials And Methods

The study was conducted in the Department of Biochemistry in collaboration with the Department of Dermatology, Regional Institute of Medical Sciences (RIMS) Hospital, Imphal, Manipur during Jan' 2008 to July'2009. It is a prospective, randomized and controlled study in which 90 vitiligo patients attending the Dermatology OPD and vitiligo clinic are enrolled in the study. Thirty five apparently healthy, age and sex matched individuals are selected to serve as control. Patients who undergone thyroid surgery and under medication for thyroid diseases, individual with pregnancy, were excluded for the study. Final diagnosis and catagorisation of the study group will be done by the concerned Dermatologists.

A prescribed proforma containing all the demographic data, revelant questionnaire were recorded for each and every case with brief clinical history suggestive of any thyroid disease as well as those referred by the clinicians.

Sample collection and processing: About 4cc of whole blood was drawn from antecubital vein from each patient as well as control, collected in sterile plain vials, sample was allowed to clot and centrifuged to separate the serum. Serum sample is stable in 2-8˚c for a maximum period of 5days and upto 30 days when stored in refrigerator at -20˚c.

In present study, all estimations were done within 3 days of collection and storage of sample was done in freezer compartment of refrigerator.

ESTIMATION OF TOTAL TRIIODOTHYRONINE (tT3):

Serum total Triiodothyronine (tT3) was estimated by Immuno-enzymometric assay using commercially available kit manufactured by Monobind Inc.Lake forest, CA 92630,USA

Intendeduse : The Quantitative Determination of Total Tri-iodothyro nine concentration in Human Serum or Plasma by a Microplate Enzyme Immunoassay (ELISA) (Gharib H et al, 1971).

ESTIMATION OF TOTAL THYROXINE(tT4)

Intended use: The Quantitative Determination of Total Thyroxine Concentration in Human Serum or Plasma by Microplate Enzyme Immunoassay (Young D S et al, 1975).

ESTIMATION OF THYROTROPIN(TSH)

Intended Use: The Quantitative Determination of Thyrotropin concentration in Human serum by a microplate Immunoenzymometric assay (Hopton M R et al, 1986).

ESTIMATION OF SERUM ANTI-THYROGLOBULIN(Anti-Tg)

Intended use: The Quantitative Determination of Thyroglobulin (Tg) Autoantibodies in Human Serum or Plasma by a Microplate Enzyme Immunoassay. Measurements of Tg autoantibodies may aid in the diagnosis of certain thyroid diseases such as Hashimoto's and Grave's as well as nontoxic goiter (Beever K, 1989).

ESTIMATION OF ANTI-THYROIDPEROXIDASE(Anti- TPO)

Intended Use

Anti-TPO is a solid phase enzyme immunoassay employing recombinant human thyroid peroxidase (TPO) from an eukaryotic expression system for the quantitative detection of antibodies against TPO in human serum. Only recombinant human antigen expressed in eukaryotic cells displays specific conformational epitopes that are accessible for human anti-TPO autoantibodies. The assay is a tool in the diagnosis of autoimmune thyroid diseases (Peter JB and Shoenfeld Y ,1996).

STATISTICAL METHOD USED:

The datas are entered, analysed and computed with SPSS version 15 software and well known statistical test like Chi-square, students' 't' test have been advocated wherever found applicable. The necessary interpretations are made. P value < 0.05 is considered significant.

III. Results And Observation

The study was conducted in the Department of Biochemistry in collaboration with the Department of Dermatology, RIMS hospital. It is a prospective, randomized & controlled study in which ninety (90) vitiligo patients attending the Dermatology OPD and vitiligo clinic are enrolled in the study. Thirty five (35) apparently healthy, age and sex matched individuals are selected to serve as control. The demographic datas such as age, sex, regional distribution, etc. and biochemical & immunological parameters such as TSH, T3, T4, anti-TG and anti-TPO are recorded. The datas are entered and computed with SPSS version 15 software and well known statistical test like Chi-square, students 't' test, etc. are used wherever found applicable and necessary.

Demographic profile

Region wise distribution

The highest number of vitiligo cases were recorded from Imphal-west district of Manipur with 33(36.67%) patients, likewise maximum number of patients with 14(40%) were also enrolled from the same district in the control group, as shown in table (1). However, the regional distribution of patients in the vitiligo group is comparable with that of the control group, with a chi-square value of 4.40 which is statistically not significant (P=0.36).

Table (1) showing the region wise distribution of patients in the two groups.

Region wise	Cases (No)	Control (No)	Chi-square value	Degree of Freedom	'P' value & Inference
Imphal-east	25(27.78%)	12(34.27%)			
Imphal-west	33(36.67%)	14(40%)			
Churachanpur	21(23.33%)	3(8.57%)	4.40	4	P=0.36
Aizawl(Mizoram)	10(11.11%)	6(17.16%)			
Kakching	1 (1.11%)	0(0%)			
Total number of patients	90(100%)	35(100%)			

(*Figure within the parenthesis indicates percentage)*

Racial/religion wise distribution

The racial/religion wise distribution of patients in the two groups are shown in table (2). Hindu contributed maximum number of patients in both the groups with 51(56.67%) & 30(85.71%) patients in the vitiligo and control groups respectively. Muslim shared 8(8.89%) & 3(8.57) patients while Christian contributed 31(34.44%) & 2(5.72) patients in the vitiligo and control groups respectively. This racial/religion wise distribution is statistically significant (P=.00) with a chi-square value of 12.11, which may be explained by the majority of patients attending the RIMS hospital are from the valley districts which are hindu dominated area.

Table (2) showing the religion wise (racial) distribution of patients in the two groups.

Religion(racial)	Cases (No)	Control (No)	Chi-square value	Degree of freedom	'P' value & Inference
Hindu	51(56.67%)	30(85.71%)			
Muslim	8(8.89%)	3(8.57)	12.11	2	P=0.00*
Christian	31(34.44%)	2(5.72%)			
Total	90(100%)	35(100%)			

*= Significant. **(***Figure within the parenthesis indicates percentage)*

Age distribution

The age ranged from 8-62 years in the vitiligo group whereas it is from 19-60 years in the control group. The age distribution, as shown in table (3), recorded an average (mean±SD) age of 29.82±14.44 and 40.06±13.59 years respectively for vitiligo and control groups with a student't' test value of 3.59, where the difference is not statistically significant (P=0.88). In the vitiligo group, maximum (13) patients are in the age range of 6-10 & 36-40 years whereas 56-above years constitute the maximum number (10) in the control group.

Sex distribution

The sex distribution, as shown in figure (4), recorded 27(30%) male & 63(70%) female patients in the vitiligo group and 13(37.14%) male & 22(62.86%) female patients in the control group respectively. This distribution is comparable with a chi-square value of 0.59 which is statistically not significant (P=0.44)
Thus, the regional distribution, religion, age and sex parameters in both the groups are equally distributed and will not affect the outcome of our study.

Subtypes of vitiligo

Seven subtypes of vitiligo are recorded in our study groups, as shown in fig. (5)**,** with maximum number of patients of 23(25.56%) each in V.vulgaris and acrofacialis subtypes while segmental has got 22(24.44%) patients. Universalis subtype has got the least with 1(1.11%) patient. Their age-wise distribution is also shown in table (6) where the distribution is statistically not significant (P>0.05) with a chi-square value of 2.99.

Age distribution

The age ranged from 8-62 years in the vitiligo group whereas it is from 19-60 years in the control group. The age distribution, as shown in table (3), recorded an average (mean±SD) age of 29.82±14.44 and 40.06±13.59 years respectively for vitiligo and control groups with a student't' test value of 3.59, where the difference is not statistically significant (P=0.88). In the vitiligo group, maximum (13) patients are in the age range of 6-10 & 36-40 years whereas 56-above years constitute the maximum number (10) in the control group.

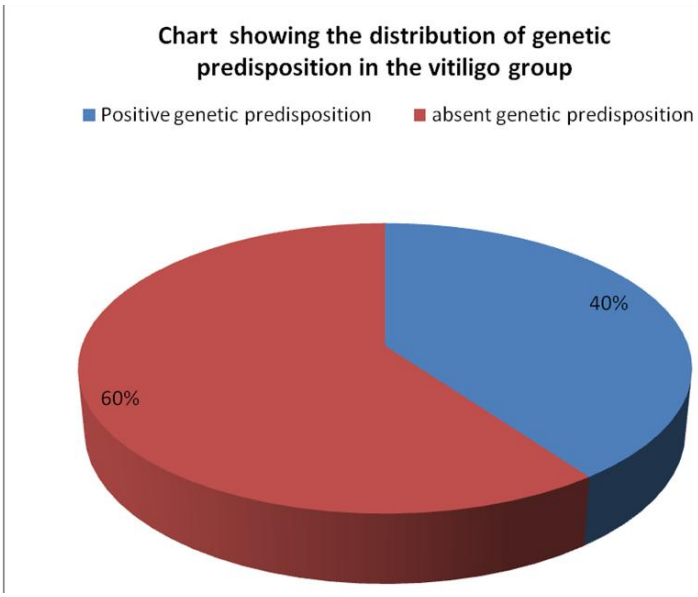

Figure (7) showing the distribution of genetic predisposition/Family history in the cases (vitiligo) group

Table (8) showing the distribution of genetic predisposition/Family history with relation to the type of vitiligo in the cases (vitiligo) group.

Types of Vitiligo	No of patients with Positive genetic predisposition	No of patients with Negative genetic predisposition	Chi-square value	'P' value & Inference
Segmental	10(11.11%)	12(13.33%)		
Vitiligo vulgaris	13(14.44%)	10(11.11%)		
Acrofacialis	10(11.11%)	13(14.44%)		
Focal	2(2.22%)	8(8.88%)		
Mucosal	0(0%)	8(8.88%)		
Mixed	1(1.11%)	2(2.22%)		
Universalis	0(0%)	1(1.11%)	5.21	P>0.05
Total	36(40%)	54(60%)		

(*Figure within the parenthesis indicates percentage)*

Biochemical assay

The thyroid hormonal assay, as shown in table (9) and (10), recorded an average (mean±SD) serum TSH, T_3 &T_4 levels of 5.70±3.12*µIU/ml*, 1.33±1.32*ng/ml* & 6.31±2.86*µg/dl* respectively in the vitiligo group while it is 4.26±2.17*µIU/ml*, 1.12±0.42 *ng/ml* & 6.81±2.48*µg/dl* respectively in the control group. The distribution in the TSH level in the two groups is statistically significant (P=0.01) with 't' test value of 2.51. However, the distribution of T_3 &T_4 levels in the groups are not significant (P>0.05).

Twentyfive (27.78%) patients have got increased serum TSH level above normal in the vitiligo group while three (8.57%) patients in the control group have got the increase value. Decreased value from the normal are noticed in 4(4.44%) patients in the vitiligo group with none in the control group. So, 61(67.78%) patients in the study group have got normal serum value while 32(91.43%) patients have normal level in the control group. There exists a significant difference in the two groups with a chi-square value of 6.41 which is statistically significant (P<0.05).

Twelve(13.33%) patients in the vitiligo group have got raised serum T_3 levels above normal while there is none in the control group. 18(20%) & 4(11.43%) patients have low level below normal in the study and control groups respectively. Normal level are noticed in 60(66.67%) and 31(88.57%) patients respectively in the study and control groups. This distribution is statistically significant (P<0.05) with a chi-square value of 5.05.

Likewise, six (6.67%) patients in the vitiligo group have got raised serum T_4 levels above normal while there is none in the control group. Low serum T_4 levels are recorded in 31(34.44%) & 6(17.14%) patients of vitiligo and control group respectively. This distribution in the T_4 level in the two groups has got a significant difference with a chi-square value of 6.42 which is statistically significant (P<0.05).

Table (9) showing the distribution of Thyroid hormonal assay in the two groups.

Thyroid hormones	Observed assay	No of patients in the Cases	No of patients in the control	Chi-square value	'P' value & Inference
TSH	*Increase TSH*	25(27.78%)	3(8.57%)	6.41	P<0.05*
	Decrease TSH				
	Normal	4(4.44%)	0(0%)		
		61(67.78%)	32(91.43%)		
	Total	90(100%)	35(100%)		
T_3	*Increase T_3*	12(13.33%)	0(0%)	5.05	P<0.05*****
	Decrease T_3	18(20%)	4(11.43%)		
	Normal	60(66.67%)	31(88.57%)		
	Total	90(100%)	35(100%)		
T_4	*Increase T_4*	6(6.67%)	0(0%)	6.42	P<0.05*****
	Decrease T_4	31(34.44%)	6(17.14%)		
	Normal	53(58.89%)	29(82.86%)		
	Total	90(100%)	35(100%)		

= Significant. (Figure within the parenthesis indicates percentage)*

Table (10) showing the distribution of thyroid hormonal and anti-immunoglobulin assay in the two groups.

Hormones/ Immunoglobulin	Cases (Mean±S.D)	Controls (Mean±S.D)	Student 't' test value	'P' value	Inference
TSH(μIU/ml)	5.70±3.12	4.26±2.17	2.51	0.01	*P<0.05**
T_4(μg/dl)	6.31±2.86	6.81±2.48	0.92	0.36	P>0.05
T_3(ng/ml)	1.33±1.32	1.12±0.42	0.92	0.36	P>0.05
AntiTPO(IU/ml)	170.18±99.49	96.42±38.45	4.2	0.00	*P<0.05**
Anti TG (IU/ml)	136.94±98.34	95.64±22.33	2.46	0.02	*P<0.05**

***= Significant**

When we study the vitiligo group, as shown in table no (11), thirty four (37.78%) patients have clinical hypothyroidism while 9(10%) patients have clinical hyperthyroidism. This difference in the distribution is statistically significant (P<0.05) with a chi-square value of 10.08.

Immunological assay

The average (mean±SD) concentration of antiTG and antiTPO, as shown in table (10), in the vitiligo group are respectively 136.94±98.34 *(IU/ml)* and 170.18±99.49 *(IU/ml)* while it is 95.64±22.33 *(IU/ml)* and 96.42±38.45 *(IU/ml)* respectively in the control group. There exists a significant difference (P=0.02 & P=0.00) in the distribution of these immunoglobulin in the two groups with student 't' test value of 2.46 and 4.2 respectively.

Seventeen(18.89%) patients have positive anti TG assay while 42(46.67%) patients are positive for anti TPO assay in the vitiligo group, as shown in table (14). 17(18.89%) patients are positive for both anti TG and anti TPO assay. Clinical thyroid disease are noticed in 7(7.78%), 17(18.89%) and 16(17.78%) patients with positive anti TG, both anti TG & anti TPO positive and anti TPO positive assay respectively in the vitiligo group. This difference in the distribution is statistically significant (P<0.05) with a chi-square value of 8.27. However, none of the patients in the control group are positive for any of the studied immunoglobulin.

Table (11) showing the distribution of patients with relation to the associated clinical thryroid dysfunction in the vitiligo (cases) group.

Thyroid dysfunction	No of patients ***with clinical*** Thyroid dysfunction	No of patients ***without clinical*** Thyroid dysfunction	Chi-square value	'P' value & Inference
Hypothyroid	34(37.78%)	47(52.22%)	10.08	P<0.05*****
Hyperthyroid	9(10%)			
Total	43(47.78%)	47(52.22%)		

= Significant. (Figure within the parenthesis indicates percentage)*

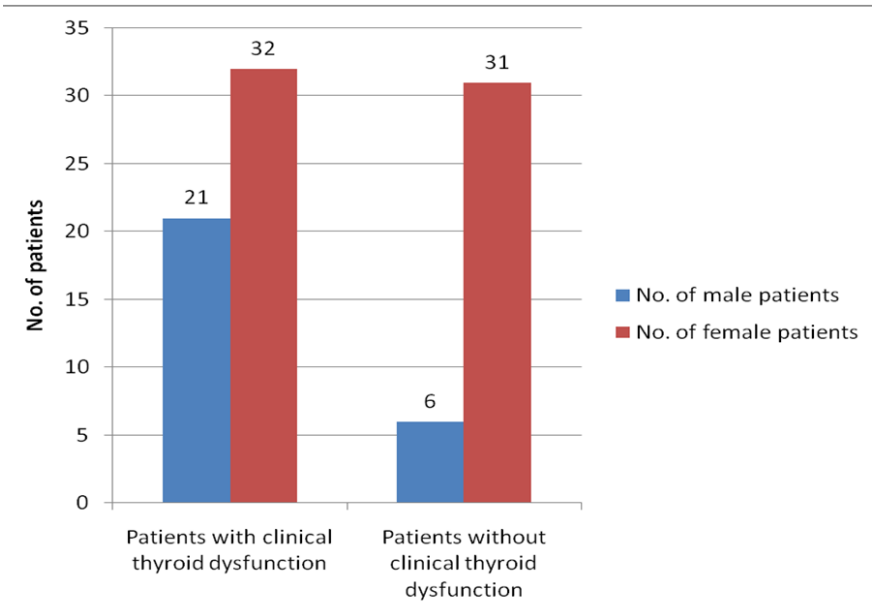

Figure (12) showing the sexwise distribution with relation to the associated clinical thryroid dysfunction in the vitiligo (cases) group.

Gender wise classification of the vitiligo group, as shown in figure (12), show 21(23.33%) male patients with clinical thyroid dysfunction while 32(35.56%) female have this feature. 31(34.44%) female & 6(6.67%) male patients does not have clinical thyroid disorder. This distribution is statistically significant (P<0.05) with a chi-square value of 5.68.

Table (13) showing the distribution of sex with relation to the associated thryroid dysfunction (clinical or any abnormal hormonal/Immunoglogical assay) in the vitiligo (cases) group.

Sex	No of patients ***with any*** Thyroid dysfunction	No of patients ***without any*** Thyroid dysfunction	Chi-square value	'P' value & Inference
Male	27(30%)	0(0%)	0.04	P>0.05
Female	52(57.78%)	11(12.22%)		
Total	79(87.78%)	11(12.22%)		

(*Figure within the parenthesis indicates percentage)*

Table (14) showing the distribution of patients with relation to the Immunoglobulin positivity and clinical thyroid diseases in the Vitiligo (Cases) group.

Immunoglobulin assay	No.of Vitiligo patients ***with*** clinical thyroid disease ***with*** positive Immunoglobulin assay	No. of Vitiligo patients ***without*** clinical thyroid disease ***with*** positive Immunoglobulin assay	Total	Chi-square value	'P' value & Inference
1. Anti TG positive assay	7(7.78%)	10(11.11%)	17(18.89%)		
2. Anti TG & anti TPO positive assay	17(18.89%)	0(0%)	17(18.89%)	8.27	P<0.05*****
3. Anti TPO positive assay	16(17.78%)	26(28.88%)	42(46.67%)		
Total	40(44.44%)	36(40%)	76(84.44%)		

= Significant. (Figure within the parenthesis indicates percentage)*

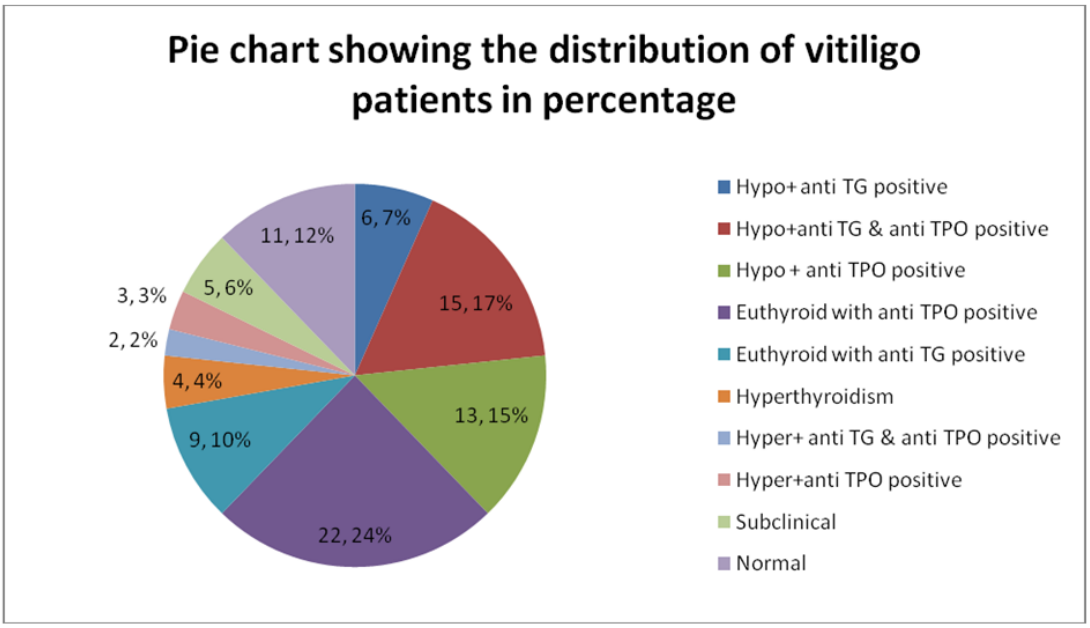

Figure (15) showing the distribution of thyroid status in the vitiligo (cases) group.

Gender wise classification of the vitiligo group, as shown in figure (12), show 21(23.33%) male patients with clinical thyroid dysfunction while 32(35.56%) female have this feature. 31(34.44%) female & 6(6.67%) male patients does not have clinical thyroid disorder. This distribution is statistically significant ($P<0.05$) with a chi-square value of 5.68.

Again, when we distribute and classify the sex of the vitiligo patients with reference to the presence of any thyroid disorder, whether clinical or biochemical or immunological, as shown in table (13), we find that 27(30%) male patients and 52(57.78%) female patients have the disorder, while only 11(12.22%) female patients does not have it. This difference in the sex distribution are statistically not significant ($P>.05$) with a chi-square value of 0.04. Thus, we can say that 11(12.22%) patients of vitiligo group have normal thyroid while the remaining 79(87.78%) patients have associated thyroid disorder.

Thus, as shown in figure (15), 11(12.22%) patients of the vitiligo group are not associated with any thyroid disorder, while the remaining 79(87.78%) patients have thyroid involment. Subclinical hypothyroidism is recorded in 5(5.56%) patients.

IV. Discussion

The study was carried out to look for any association of vitiligo and thyroid dysfunction with autoimmune thyroid disease and to find out clinical characteristics of vitiligo, which may predict such an association. It is a prospective, randomized and controlled study in which 90 vitiligo patients attending the Dermatology OPD and vitiligo clinic of RIMS Hospital, in period of Jan'2008 - July'2009 were enrolled for this study. Besides recording the age, sex, regional distribution, clinical features of vitiligo and thyroid disease, antithyroid autoantibody assays (anti-thyroglobulin, anti-TPO) and thyroid hormone profiles were done in these cases and 35 appropriately age and sex matched controls.

The highest vitiligo cases were observed from Imphal- west district of Manipur with 33 (36.67%) patients and 14 (40%) control group as shown in table 1, with a chi- square value of 4.40 which is not significant statististically ($P=0.36$). However, there is no correlation of regional distribution of maximum patients and vitiligo, reason being the location of hospital in this region, followed by Imphal-East: 25 (27.78%) cases, 12 (34.27%) controls, then Churachandpur-21 (23.33%) cases, 3 (8.57%) controls which are situated abit farther away from RIMS hospital. Vitiligo, first noted in approximately 1500BC, afflicts all populations around the world with diverse prevalence rates among different geographic regions and ethnic groups ranging from 0.1% to 2% in the studies of Majumder PP et al, 2000.

As to the racial/ religion wise distribution, depicted in table 2, Hindu contributed maximum number of patients in both groups with 51 (56.67%) and 30 (85.71%) patients in vitiligo and control groups respectively as compared to other religions. Muslim shared 8 (8.89%) cases and 3 (8.57%) controls, whereas Christian contributed 31 (34.44%) cases and 2 (5.72%) control. The difference between Hindus and other religions is statistically significant ($P=.00$) with a chi-square value of 12.11, which may be explained by the fact that majority of patients attending the RIMS Hospital are from the Hindu dominated valley districts. There are similar studies conducted in the dark races especially from the Indian subcontinent. Shyria D et al (2003) have attempted to demonstrate the association of vitiligo with thyroid disease in South Indian population where the frequency of thyroid dysfunction is high.

As an important feature of complex diseases, the age of disease onset has routinely been analyzed in association studies. The mean ages of onset of vitiligo that we observed at about 8 years were almost identical to those found by Norlund JJ et al (1997) and Majumder PP et al (1993). Our findings were also in conformity with the findings of by Roth C et al (1994). However, not all, patients with segmental vitiligo and negative family history were afflicted at about age 15 years as described by Hann SK and Lee HJ (1996). Previous studies by Zhang XJ et al (2004) in their analysis of probands showed an equal distribution among the sexes with mean age of 22.89±13.26years. The age distribution , as shown in table (3), ranged from 8-62 years in vitiligo group, with an average (mean±SD) age of 29.82±14.44 years. Maximum (13 patients) are in the age range of 6-10 and 36-40 years.

Previous studies were inconsistent as to whether male and female patients were affected by vitiligo with equal frequency. In this study, there were 63 (70%) female vitiligo patients as compared to 27 (30%) male patients. This is in agreement with the findings of Lacovelli P et al (2003) but in contrast with the findings of Shyria D et al (2003) and Zhang XJ et al (2004) where equal distribution among the sexes were reported. However, prevalence distributions might be different in different ethnic groups. In my opinion, the differences probably result from: variable factors underlying vitiligo in different ethnic groups, fewer participants in the study, or preponderance of female respondents in this region where the study is taken up.

In this study patients were classified according to their clinical manifestations by expert dermatologists of RIMS hospital. Seven subtypes of vitiligo are recorded in the study group, as depicted in figure 5, along with age wise distribution in table 6, with maximum number of patients of 23 (25.56%) each in V.vulgaris and acrofacialis subtype while segmental has got 22 (24.44%) patients. Universalis subtype has got the least with 1 (1.11%) patient which is same as findings of Zhang XJ et al (2004) who also reported vitiligo vulgaris as maximum among their study group. It can be inferred that ages of vitiligo onset were closely associated with patients' clinical phenotypes. Age of onset for universalis vitiligo is the least, which may be an indicative of an important environmental factor underlying its etiopathogenesis. However, it needs verification in future study

V. Conclusion

The study was conducted in the Department of Biochemistry in collaboration with the Department of Dermatology, RIMS Hospital. It is a prospective , randomized and controlled study in which 90 vitiligo patients attending the Dermatology OPD and vitiligo clinic are enrolled in the study. Thirty five apparently healthy, age and sex matched individuals are selected to serve as control. The demographic datas such as age, sex, regional distribution , etc and biochemical and immunological parameters such as TSH,T_3 ,T_4 and anti-TG, anti-TPO are recorded. The datas are entered and computed with SPSS version 15 software and well known statistical test like Chi-square, students 't' test , etc. are used wherever found applicable and necessary.

The highest vitiligo cases were observed from Imphal-West district of Manipur with 33 (36.67%) patients followed by Imphal- East 25 (27.78%) cases, and Churachandpur 21 (23.33%) cases, which are situated abit farther away from RIMS Hospital.

Religionwise, Hindus contributed maximum number of patients (56.67%). The difference in prevalence rate among different religion is statistically significant with P=.00 and chi-square value 12.11.

The minimum age of onset of vitiligo observed in the study is 8 years, proving early onset of the disease in children and adolescents age ranged from 8-62 years in vitiligo group with a mean age of 29.82±14.44 years. The disease is more prevalent in female (70%) as compared to male (30%).

Distribution of subtypes of vitiligo with relation to genetic predisposition is statistically significant (P<0.05) with chi-square value (5.21) except for mucosal and universalis subtypes, indicative of a multifactorial causes of vitiligo.

This study shows an association of vitiligo with autoimmune thyroid disease along with clinical thyroid diseases. Clinical hypo- thyroidism (37.78%) is predominant over clinical hyperthyroidism (10%), which is statistically significant (P<0.O5). Females (35.56%) are having clinical thyroid dysfunction more than males (23.33%).

It is observed that, 87.78% patients have thyroid involvement, whether clinical or biochemical or immunological. Out of which 5.56% is subclinical hypothyroidism. Only 12.22% of vitiligo patients are not associated with any thyroid disorder.

The prevalence of autoimmune thyroid disease (AID) with thyroid immunoglobulin antibodies like anti-TPO is highest among vitiligo patients (46.67%) followed by both positive anti-TG and anti-TPO

(18.89%) and only positive anti-TG (18.89%) which is statistically significant indicating autoimmune thyroid disorders are frequently associated with vitiligo (46%).

Nonetheless, there exist a correlation between the extent of depigmentation and level of vitiligo antibodies.

There is increased incidence of autoimmune thyroid diseases among vitiligo patients. Various thyroid antibodies were detected in thyroid disorders, suggesting that these thyroid antibodies could act as sensitive markers for detection of early and subclinical autoimmune disorders of thyroid gland including Graves' disease and Hashimoto's thyroiditis. The reason being vitiligo usually precedes the onset of thyroid dysfunction.

Therefore, these thyroid autoantibodies specially anti-TPO, anti-TG should be included along with thyroid parameters TSH, T_3,T_4 in routine investigation of vitiligo, as association of vitiligo with autoimmune thyroid disease is very much common in the North - Eastern region of India.

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