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*RESEARCH ARTICLE*

# SCREENING OF THYROID AUTOANTIBODIES ANTI-TG, ANTI-TPO AND ITS CORRELATION WITH CLINICAL THYROID DISEASES IN VITILIGO IN THE NORTH-EASTERN REGION OF INDIA

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## Abstract

**Background and aims:** Vitiligo is an acquired sometimes familial depigmentary disorder of the skin and hair that results from selective destruction of melanocytes or pigment cells. 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 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 thyroid diseases in vitiligo patients.

**Methods:**It is a prospective, randomized and controlled study. 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. A prescribed proforma containing all the demographic data, relevant 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.interpretationsare made. P value < 0.05 is considered significant. The Quantitative Determination of Thyroglobulin (Tg) Autoantibodies in Human Serum or Plasma by a Microplate Enzyme Immunoassay.

**Results:**Vitiligo group, 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. 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.

**Conclusion:**There is increased incidence of autoimmune thyroid diseases among vitiligopatients. Various thyroid antibodies were detected in thyroid disorders, suggesting that these thyroid antibodies could act as sensitive markers for detection of early

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and subclinical autoimmune disorders of thyroid gland including Graves' disease and Hashimoto's thyroiditis. The reason beingvitiligo 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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## Introduction:-

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. 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. In vitiligo, recent findings shows increased prevalence of autoantibodies which includes thyroid peroxidase antibody (anti-TPO) and thyroid globulin antibody (anti-TG).

Patients and animal with vitiligo have antibodies, "vitiligo antibodies" to human melanocytes that can be demonstrated by immunoprecipitation assays or by indirect immunofluorescense technics with the use of fresh frozen skin as substrate. They are uncommon in normal individuals or in patients with nonpigmentary skin diseases. A remarkable correlation between the level of serum vitiligo antibodies and the extent of depigmentation. These antibodies were detected in 50% of patients with minimal type vitiligo (<2% body surface involvement), but 93% in more extensive type (>5% surface involvement). This observation strongly support the concept that vitiligo is an autoimmune disorder.

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 needs 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. Therefore, the present study is taken up to evaluate the prevalence of thyroid 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.

In patients with autoimmune diseases, the prevalence of a clinical syndrome attributable to the circulating antibodies is low and their presence is presumed to reflect excessive stimulation of B-lymphocyte clones with autoreactive potential and present in almost all patients with Hashimoto's thyroiditis, in 2/3 of patients with post partum thyroiditis. Also, 75% patients with Graves' hyperthyroidism.

Autoimmune thyroid disease (AITD) is the most organ specific autoimmune disorder usually resulting in dysfunction (hyper or hypofunction of thyroid gland. The syndromes comprising auto immune thyroid disease and many intimately related illnesses: Graves' disease with goiter, hyperthyroid and associated ophthalmopathy, Hashimoto's thyroiditis with goiter and euthyroidism or hypothyroidism also thyroid dysfunction occurs independently of pregnancy and in 5-6% of postpartum women and thyroiditis induced by different drugs and other environmental influences. The syndrome are connected together by their similar thyroid pathogens, similar immune mechanisms, co-occurrence in family groups, transition from one

clinical picture to another within the same individual over time. Thyroid peroxidase, TPO, the primary enzyme involved in thyroid homonogenesis, was initially identified in 1959 as 'thyroid microsomal antigen'. It is uncertain whether TPO autoantibodies or TPO-specific T cells are the primary cause of thyroid inflammation, which can lead to thyroid failure and hypothyroidism. TPOAbs are hallmark of AITB and present in almost all patients with Hashimoto's thyroiditis, in 2/3 of patients with post partum thyroiditis. Also, 75% patients with Graves' hyperthyroidism.

Skin diseases in general and vitiligo in particular may have profound psychological and social effects on the sufferers. Studies have demonstrated the quality of life impairment caused by vitiligo, a disfiguring skin disease affecting at least 1% of the population. Many vitiligo patients are distressed, especially in relation with social encounters, or feel embarrassed when exposing the body. Moreover, vitiligo patients may suffer from low self- esteem and poor body image, experience discrimination from others and feel stigmatized. One of the main objectives of patient-oriented care is to reduce the psychosocial burden and quality of life impairment caused by the disease. Improvement of quality of life is related to a patient's satisfaction with care, both may have a positive effect on treatment adherence or outcome. Various available treatment options aim to inhibit further progress of the disease and induce repigmentation. Few studies have assessed the effect of treatment on the quality of life.

## 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 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).

**Principle**

**A sequential ELISA Method**

The reagents required for the sequential ELISA assay include immobilized antigen, circulating autoantibody and enzyme-linked species-specific antibody. In this procedure, the immobilization takes

place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated thyroglobulin antigen.

Upon mixing biotinylated antigen and a serum containing the autoantibody, reaction results between the antigen and the antibody to form an immune-complex. The interaction is illustrated by the following equation:

$$h\text{-}Ab_{(x\text{-}Tg)} + {}^{Btn}Ag_{(Tg)} \underset{k_{-a}}{\overset{k_a}{\leftrightharpoons}} h\text{-}Ab_{(x\text{-}Tg)} - {}^{Btn}Ag_{(Tg)}$$

${}^{Btn}Ag_{(Tg)}$ = Biotinylated Antigen ( Constant Quantity)
$h\text{-}Ab_{(x\text{-}Tg)}$ = Human Auto- Antibody (Variable Quantity)
$Ab_{(x\text{-}Tg)}\text{-}{}^{Btn}Ag_{(Tg)}$ = Immune complex (Variable Quantity)
$k_a$ = Rate Constant of Association
$k_{-a}$ = Rate Constant of Disassociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antigen. This interaction is illustrated below:

$h\text{-}Ab_{(x\text{-}Tg)}\text{-}{}^{Btn}Ag_{(Tg)} + Streptavidin_{c.w.} \Rightarrow$ immobilized complex(IC)
$Streptavidin_{c.w.}$ = Streptavidin immobilized on well
Immobilized complex (IC) = sandwich complex bound to the solid surface

After the incubation time, the well is washed to separate the unbound components by aspiration and /or decantation. The enzyme linked species-specific antibody (anti-h-IgG) is then added to the microwells.

This conjugates binds to the immune complex that formed.

$$I.C_{(h\text{-}IgG)} + {}^{Enz}Ab_{(X\text{-}h\text{-}IgG)} \Rightarrow {}^{Enz}Ab_{(X\text{-}h\text{-}IgG)} - I.C._{(h\text{-}IgG)}$$

$I.C_{(h\text{-}IgG)}$ = Immobilised immune complex (Variable Quantity)
${}^{Enz}Ab_{(X\text{-}h\text{-}IgG)}$ = Enzyme-antibody Conjugate( Constant quantity)
${}^{Enz}Ab_{(X\text{-}h\text{-}IgG)} - I.C._{(h\text{-}IgG)}$ = Ag-Ab Complex (Variable Quantity)

The anti-h-IgG enzyme conjugate that binds to the immune complex in a second incubation is separated from unreacted material by a wash step. The enzyme activity in this fraction is directly proportional to the antibody concentration in the specimen. By utilizing several different serum references of known antibody activity, a reference curve can be generated from which the antibody activity of an unknown can be ascertained (Mak T, 1994).

**Specimen Collection And Preparation**

The specimen shall be serum or plasma separated from a venipuncture collected blood sample. For accurate comparision to established normal values, a fasting morning serum sample is obtained, blood collected in a plain redtop venipuncture tube without additives or anti-coagulant containing EDTA or heparin. Centrifufe the specimen for separating serum or plasma from the cells.

Samples are refrigerated at 2-8˚C for maximum period of 5 days. If the specimen cannot be assayed within this time, store at -20˚C for upto 30 days. Avoid repetitive freezing and thawing . When assayed in duplicate , 0.100 ml of diluted sample is required.

**Sensitivity**

The anti-TgAccubind$^{TM}$ELISA has a sensitivity of 5IU/ml.

**Specificity**

Interferences from ANA, DNA, thyroid peroxidase (TPO) and rheumatoid antibodies were found to be insignificant in the assay system (Vole R, 1994).

**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.

**Principle Of The Assay And Clinical Application**
Thyroid peroxidase (TPO) is a large (105kDa) membrane –bound glycoprotein of thyroid gland. It is the major enzyme involved in multiple steps of thyroid hormone synthesis.

TPO is one out of three major thyroid autoantigens besides thyroglobulin (Tg) and the TSH-receptor. The presence of auto antibo-dies to TPO and Tg today is an established tool for diagnosing chronic autoimmune thyroiditis as well as for the differential diagnosis of hypothyroidism including its subclinical and latent type (Portmann L et al, 1985).

**Principle Of The Test**
Serum samples diluted in the ratio 1:101, are incubated in the microplates coated with the specific antigen. Patient's antibodies, if present in the specimen, bind to the antigen. The unbound fraction is washed off in the following step. Afterwards anti-human immunoglobulins conjugated to horseradish peroxidase (conjugate) are incubated and react with the antigen-antibody complex of the samples in microplates. Unbound conjugate is washed off in the following steps. Addition of TMB-substrate generates an enzymatic colorimetric (blue) reaction, which is stopped by diluted acid (color changes to yellow). The rate of color formation from the chromogen is a function of the amount of conjugate bound to the antigen-antibody complex and this is proportional to the initial concentration of the respective antibodies in the patient sample.

**Storage and Shelf Life**
All reagents and the microplate are stored at 2-8˚C/35-46˚F, in their original containers. Once prepared, reconstituted solutions are stable for 1 month at 2-8˚C/35-46˚F, at least.

**Sample Collection, Handling and Storage**
Freshly collected serum samples are used. Blood withdrawal must follow national requirements.

Not to use icteric, lipemic, hemolysed or bacterially contaminated samples. Sera with particles should be cleared by low speed centrifugation (<1000 x g). Blood samples should be collected in clean, dry and empty tubes. After separation, the serum samples should be used immediately, respectively stored, tightly closed at 2-8˚C/35-46˚F up to three days or frozen at -20˚C/-4˚F for longer periods.

**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

## Results:-

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, $T_3$, $T_4$, 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.

**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<br>Imphal-west<br>Churachanpur<br>Aizawl(Mizoram)<br>Kakching | 25(27.78%)<br>33(36.67%)<br>21(23.33%)<br>10(11.11%)<br>1 (1.11%) | 12(34.27%)<br>14(40%)<br>3(8.57%)<br>6(17.16%)<br>0(0%) | 4.40 | 4 | P=0.36 |
| Total number of patients | 90(100%) | 35(100%) | | | |

**(**Figure within the parenthesis indicates percentage) Racial/religion wise distribution

**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<br>Muslim<br>Christian | 51(56.67%)<br>8(8.89%)<br>31(34.44%) | 30(85.71%)<br>3(8.57)<br>2(5.72%) | 12.11 | 2 | P=0.00* |
| Total | 90(100%) | 35(100%) | | | |

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

**Table 3:-** 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<br>Imphal-west<br>Churachanpur<br>Aizawl(Mizoram)<br>Kakching | 25(27.78%)<br>33(36.67%)<br>21(23.33%)<br>10(11.11%)<br>1 (1.11%) | 12(34.27%)<br>14(40%)<br>3(8.57%)<br>6(17.16%)<br>0(0%) | 4.40 | 4 | P=0.36 |
| Total number of patients | 90(100%) | 35(100%) | | | |

**(**Figure within the parenthesis indicates percentage) Racial/religion wise distribution

**Table 4:-** 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<br>Muslim<br>Christian | 51(56.67%)<br>8(8.89%)<br>31(34.44%) | 30(85.71%)<br>3(8.57)<br>2(5.72%) | 12.11 | 2 | P=0.00* |
| Total | 90(100%) | 35(100%) | | | |

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

**Table 5:-** 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<br>Imphal-west<br>Churachanpur<br>Aizawl(Mizoram)<br>Kakching | 25(27.78%)<br>33(36.67%)<br>21(23.33%)<br>10(11.11%)<br>1 (1.11%) | 12(34.27%)<br>14(40%)<br>3(8.57%)<br>6(17.16%)<br>0(0%) | 4.40 | 4 | P=0.36 |
| Total number of patients | 90(100%) | 35(100%) | | | |

**(**Figure within the parenthesis indicates percentage) Racial/religion wise distribution

**Table 6:-** 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)

**Table 7:-** Showing the distribution of vitiligo types with relation to the age in the cases group.

| Age Range(in years) | Segmen-tal | Vitiligo vulgaris | Acrofa-Cialis | Focal | Mucosal | Mixed | Univer-salis | Statistical test | 'P' value |
|---|---|---|---|---|---|---|---|---|---|
| 6-10 | 2(2.22%) | 3(3.33%) | 2(2.22%) | 2(2.22%) | 1(1.11%) | 0(0%) | 0(0%) | | |
| 11-15 | 1(1.11%) | 2(2.22%) | 0(0%) | 0(0%) | 1(1.11%) | 1(1.11%) | 0(0%) | | |
| 16-20 | 3(3.33%) | 0(0%) | 3(3.33%) | 2(2.22%) | 1(1.11%) | 0(0%) | 0(0%) | | |
| 21-25 | 2(2.22%) | 2(2.22%) | 0(0%) | 0(0%) | 1(1.11%) | 0(0%) | 0(0%) | | |
| 26-30 | 2(2.22%) | 1(1.11%) | 3(3.33%) | 0(0%) | 1(1.11%) | 0(0%) | 0(0%) | | |
| 31-35 | 3(3.33%) | 2(2.22%) | 2(2.22%) | 2(2.22%) | 1(1.11%) | 0(0%) | 0(0%) | | |
| 36-40 | 3(3.33%) | 2(2.22%) | 5(5.55%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | Chi-square value of 2.99 | P>0.05 |
| 41-45 | 2(2.22%) | 1(1.11%) | 2(2.22%) | 2(2.22%) | 0(0%) | 0(0%) | 0(0%) | | |
| 46-50 | 1(1.11%) | 4(4.44%) | 4(4.44%) | 0(0%) | 2(2.22%) | 1(1.11%) | 0(0%) | | |
| 51-55 | 2(2.22%) | 2(2.22%) | 1(1.11%) | 1(1.11%) | 0(0%) | 0(0%) | 1(1.11%) | | |
| 56& above | 1(1.11%) | 4(4.44%) | 1(1.11%) | 1(1.11%) | 0(0%) | 1(1.11%) | 0(0%) | | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Total | 22 | 23 | 23 | 10 | 8 | 3 | 1 | | |

**(**Figure within the parenthesis indicates percentage)

**Table 8:-** 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<br>Decrease TSH<br>Normal | 25(27.78%)<br><br>4(4.44%)<br><br>61(67.78%) | 3(8.57%)<br><br>0(0%)<br><br>32(91.43%) | 6.41 | P<0.05* |
| | Total | 90(100%) | 35(100%) | | |
| $T_3$ | Increase $T_3$<br>Decrease $T_3$<br>Normal | 12(13.33%)<br>18(20%)<br>60(66.67%) | 0(0%)<br>4(11.43%)<br>31(88.57%) | 5.05 | P<0.05**** |
| | Total | 90(100%) | 35(100%) | | |
| $T_4$ | Increase $T_4$<br>Decrease $T_4$<br>Normal | 6(6.67%)<br>31(34.44%)<br>53(58.89%) | 0(0%)<br>6(17.14%)<br>29(82.86%) | 6.42 | P<0.05**** |
| | Total | 90(100%) | 35(100%) | | |

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

**Table 9:-** 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**

**Table 10:-** 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<br><br>Hyperthyroid | 34(37.78%)<br><br>9(10%) | 47(52.22%) | 10.08 | P<0.05**** |
| Total | 43(47.78%) | 47(52.22%) | | |

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

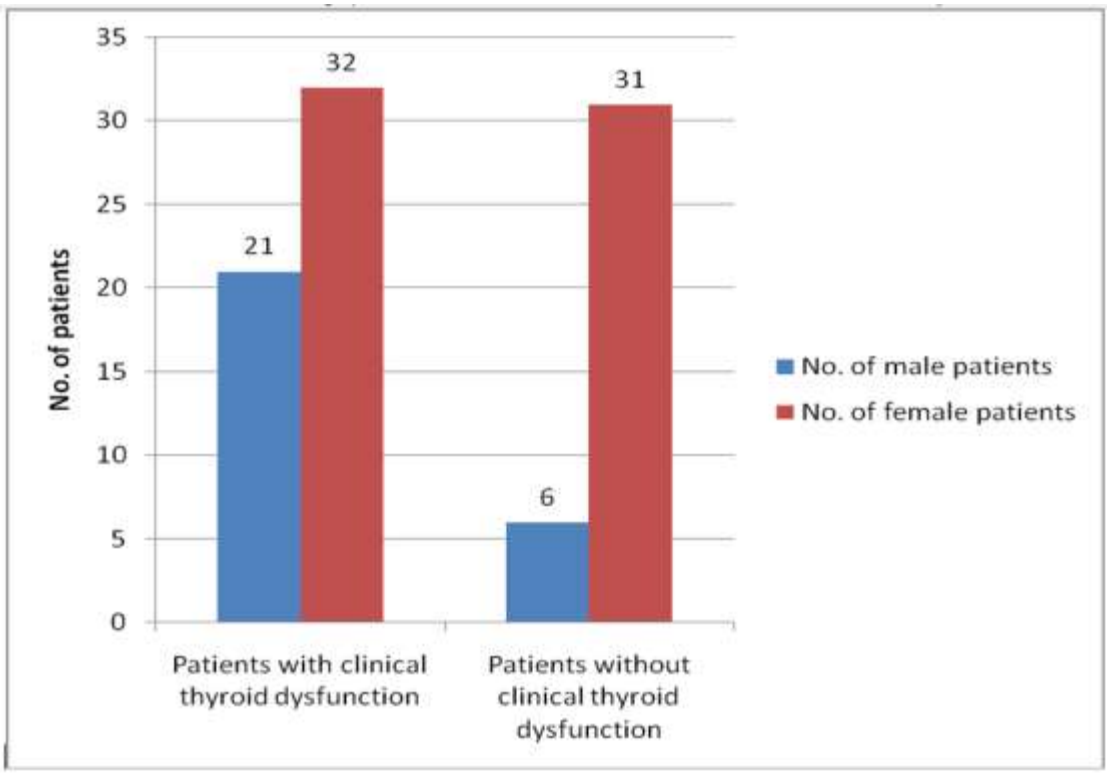


**Figure 1:-** Showing the sexwisedistribution with relation to the associated clinical thryroid dysfunction in the vitiligo (cases) group.

**Table 11:-** 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 12:-** Showing the distribution of patients with relation to the Immunoglobulin positivity and clinical thyroid diseases in the Vitiligo (Cases) group.

| Immunoglobulin assay | No.ofVitiligo 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 | 17(18.89%) | 0(0%) | 17(18.89%) | 8.27 | P<0.05***** |

| | | | | | |
|---|---|---|---|---|---|
| positive assay<br>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)

## 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, 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%.

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 who 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. However, not all, patients with segmental vitiligoand negative family history were afflicted at about age 15 years. Previous studies 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. 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.

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## 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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