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**RESEARCH ARTICLE**

# STUDY ON PREVALENCE OF ANTI-CCP ANTIBODIES IN PATIENTS WITH NEWLY DIAGNOSED AUTOIMMUNE SUBCLINICAL HYPOTHYROIDISM

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**ABSTRACT**

Subclinical Hypothyroidism (SCH) is a common disorder with a prevalence of 1–10% in the adult population. Aetiologically, most cases of persistent SCH are due to autoimmune thyroiditis (AIT). Hypothyroidism and inflammatory arthritis tend to coexist, but data on this association are sparse. Rheumatoid arthritis (RA) is one of the most common autoimmune diseases and also the most frequent chronic inflammatory arthropathy. Anti cyclic-citrullinated-peptide (anti-CCP) antibodies hold promise for earlier and more accurate diagnosis of disease, improved prognostic information, and have been implicated in RA pathogenesis. In the present study, newly diagnosed subclinical hypothyroid patients tested positive for either anti TPO or anti TG antibodies were screened for the presence of anti- CCP by ELISA. 20% of patients showed the presence of anti CCP antibodies while controls consisting of non autoimmune subclinical hypothyroid patients showed only 2.5%. Moreover, the prevalence of anti-CCP antibodies was more in female population (75%) compared to male patients similarly in controls the positivity was observed only in female. The healthy euthyroid showed absence of anti CCP antibodies. The study thus emphasizes the necessity for screening the patients with autoantibodies like anti TPO & anti TG for inflammatory arthritis such as Rheumatoid arthritis.



## INTRODUCTION

Subclinical hypothyroidism is defined biochemically as a normal serum free thyroxine (T4) concentration in the presence of an elevated serum thyroid-stimulating hormone (TSH) concentration. The incidence of SCH varies between 4 and 10% depending upon the gender, age and population studied (Canaris *et al* 2000; Vanderpump *et al* 1995). The consequences of SCH are variable at several levels and may depend on the duration and the degree of elevation of the serum TSH. (Surks *et al* 2004).

Most cases of persistent SCH are due to autoimmune thyroiditis (AIT); however, germline loss of function mutations in the TSH receptor account for a small proportion of cases (Alberti *et al* 2002). Hypothyroidism and inflammatory arthritis tend to coexist, but data on this association are sparse. Rheumatoid arthritis (RA) is one of the most common autoimmune diseases and also the most frequent chronic inflammatory arthropathy. The disease affects around 1% of the world population, 75% of which are female. It is characterised by inflammation of the synovial membrane, which spreads symmetrically from the small to large joints leading to the destruction of the joints in the late phase accompanied by a systemic involvement of the soft tissue.(Gabriel *et al* 2001).The diagnosis of RA is established primarily on clinical criteria and serologic findings. Historically, rheumatoid factor (RF), which is an antibody specific for the Fc portion of human IgG, has been considered a marker for RA. (Banal *et al* 2009).

Serological testing for rheumatoid factor is complicated by moderate sensitivity and specificity, and high rates of positivity in other chronic inflammatory and infectious diseases such as Sjogren's syndrome and chronic viral hepatitis.(Dorner *et al* 2004).Several studies have shown that anti-perinuclear autoantibodies, otherwise known as anti-keratin autoantibodies, are found in patients with RA.(Vincent *et al* 1999) It has been discovered that these antibodies recognize an epitope that contains the deamidated form of arginine called citrulline. Enzyme-Linked Immunosorbent Assay (ELISA) testing for these autoantibodies directed against anti-cyclic citrullinated

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peptide (anti-CCP) is reasonably sensitive and highly specific in patients with RA (Schellekens *et al* 2000).The pathogenesis of anti-CCP antibodies in rheumatoid arthritis has been shown to be attributable to the body's humoral response to citrulline. Anti-CCP antibodies hold promise for earlier and more accurate diagnosis of disease, improved prognostic information, and have been implicated in RA pathogenesis. (Niewold *et al* 2007). Relation between rheumatoid arthritis (RA) and the thyroid gland have been studied extensively for a long time. Several studies are available reporting the relation between autoimmune thyroid disease in RA but not the same viceversa. Hence this study was designed to study the prevelance of anti CCP in newly developed autoimmune subclinical hypothyroid patients.

## MATERIALS AND METHODS

Patients were screened for thyroid disorder by measuring their f T3,f T4,TSH by direct chemiluminescence technique (ADVIA Centaur XP, USA).Clearance was obtained from Institute Ethical Committee ( IEC NO:0169/S/2014) prior to the commencement of study. The patients with normal f T3,f T4 and raised TSH ((above 5.5 µIU/ml) were catagorised into subclinical hypothyroid patients who were newly diagnosed. (Ambika *et al* 2013).The samples were then tested for the presence of antithyroid antibodies (anti-TPO and anti-Tg) using Biochip technology. Serum samples were diluted in 1:10 dilution with Phosphate buffer saline and Tween20 and vortexed. Samples were applied to the reaction fields of the reagent tray. The biochip slides consist of a substrate combination of Thyroid gland (monkey) and Kidney (rat). The samples which showed characteristic pattern with thyroid gland were considered positive for antithyroid antibodies.

30µl of diluted sample was applied to each reaction field of the reagent tray without any air bubbles. The Biochip slide with the biochip combination of HEp 2010 and Primate Liver was fitted into the corresponding recesses of the reagent tray. The samples were incubated at room temperature for 30 minutes. Then the Biochips were rinsed with a flush of PBS-tween using a beaker and then it was immersed in a coupling jar containing PBS-tween for 5 minutes. 25µl of fluorescein-labelled anti-human globulin was added to each reaction field of a clean titer plane. Then Biochip slide was removed from the coupling jar and within five seconds the back and the long sides of the Biochip slide was blotted with a paper towel. The Biochip slide was immediately put into the recesses of the reagent tray. Again the biochip was incubated at room temperature for 30 minutes. The Biochip slides were rinsed again with a flush of PBS-tween using a beaker and then it was immersed in a cuvettes containing PBS-tween for 5 minutes. 10µl of glycerol was placed onto a cover glass. The biochip was removed from the cuvette and it was dried. The biochip facing downwards was put onto the cover glass. The fluorescence was read with the microscope initially in objective 20X. And focused using 40X. (Euroimmun, Germany).

## RESULTS

The study involved forty newly diagnosed autoimmune subclinical hypothyroid patients with 11 males(28%)and 29 females(72%).The average age was 45yrs. Controls involved forty non autoimmune subclinical hypothyroid patients .The presence of anti CCP was found in eight samples which accounts to around 20% whereas:. in the controls it was only 2.5%. The study was extended to taking gender into consideration, females showed higher prevalence of around 75% and males around 25%, while in controls positivity was only in female patients.

**Table1** Prevalence of anti- CCP antibody in autoimmune subclinical hypothyroid patients and subclinical hypothyroid patients

| s.no | Category | Total no. of samples | No. of +ve samples | % | Male | Female |
|---|---|---|---|---|---|---|
| 1 | I | 40 | 1 | 2.5 | - | 1 |
| 2 | II | 40 | 8 | 20 | 2 | 6 |

Note: I- Subclinical Hypothyroid patients, II -Autoimmune Subclinical Hypothyroid patients.

**Table 2** Prevalence of anti -CCP antibodies in female patients

| s.no | Catagory | Total no. of patients | No. of female patients | Total no. Of positive patients | No. of positivity | % |
|---|---|---|---|---|---|---|
| 1 | I | 40 | 20 | 1 | 1 | 100 |
| 2 | II | 40 | 29 | 8 | 6 | 75 |

Note: I- Subclinical Hypothyroid patients, II -Autoimmune Subclinical Hypothyroid patients.

**Table3** Prevalence of anti -CCP antibodies in male patients

| s.no | Catagory | Total no. of patients | No. of male patients | Total no. Of positive patients | No. of positivity | % |
|---|---|---|---|---|---|---|
| 1 | I | 40 | 20 | 2 | 0 | 0 |
| 2 | II | 40 | 11 | 8 | 2 | 25 |

Note: I- Subclinical Hypothyroid patients, II -Autoimmune Subclinical Hypothyroid patients.

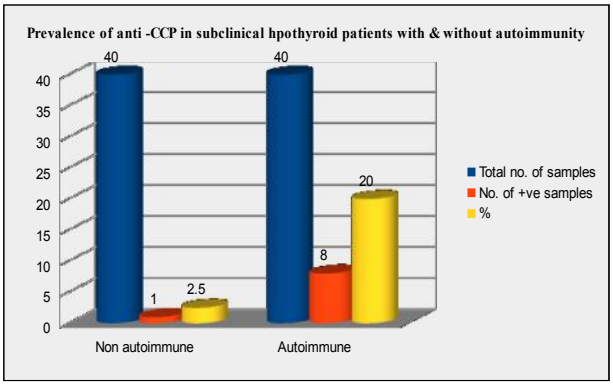


**Fig 1**Prevalence of anti- CCP antibody in autoimmune Subclinical hypothyroid patients and subclinical hypothyroid patients

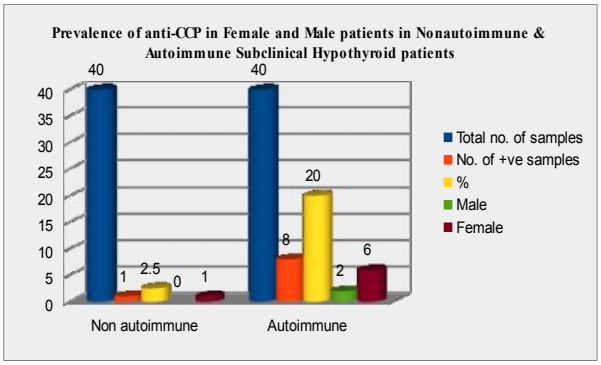


**Fig 2** Prevalence of anti -CCP antibodies in Male patients & Female patients

## DISCUSSION

The coexistence of thyroid dysfunction and RA has been a subject of debate (Delamere *et al* 1982). Abnormal or changing thyroid status may precipitate or exacerbate musculoskeletal disease, especially when common features and symptoms for hypothyroidism such as fatigue, malaise, dyslipidemia, and increased weight could be masked by the original RA symptoms (Porkodi *et al* 2004). Some workers claim that subclinical hypothyroidism is present in 9.4%–21% of patients with RA(Dołęga *et al* 1996; Arasini *et al* 2005; Dessein *et al* 2004). Autoimmune diseases such as RA may accelerate the progression of subclinical disease into a clinical disease, which might explain the lower prevalence of subclinical hypothyroidism in RA and the higher prevalence of hypothyroidism in female RA patients in comparison with women of the general population (Chan *et al* 2001).Similarly, autoimmune thyroid disease (AITD) is reported in up to 30% of patients with RA. (Peter *et al* 2011).Studies by Fabiola Atzeni *et al*., report increased prevalence of anti-thyroid antibodies in RA patients with a low prevalence of hormonal alterations (Fabiola Atzeni *et al* 2008).

A relatively high frequency of anti-CCP antibodies has also been reported in patients with non-RA connective tissue disorders such as systemic lupus erythematosus (SLE; 15%), Sjögren's syndrome (SS; 14%), polymyositis/ dermatomyositis (23%), and Scleroderma (16%). The median level of anti-CCP antibodies, however, was lower in these non-RA patients (7–35units per ml) compared to patients with RA (100 units per ml or more (Matsui *et al* 2006).Studies by Przygodzka report higher prevalence of ATD in female RA patients compared with controls which indicates the need for screening not only of thyroid function, but also of the presence of anti-TPO antibodies as the ATD marker in RA patients. Their presence does not correlate with the occurrence of thyroid disorders in RA patients. Monitoring of thyroid function is of particular importance since as already shown the course of thyroid disease in RA patients is often asymptomatic (Przygodzka *et al* 2009). But studies to evaluate the prevalence of RA markers in autoimmune hypothyroid patients are sparse(Matsui *et al* 2006). This study was designed to evaluate the prevalence of anti-CCP, the diagnostic marker for RA in autoimmune subclinical hypothyroid patients. It included 50 newly diagnosed autoimmune subclinical hypothyroid patients with 26 females and 24 males.

The prevalence of anti CCP antibodies were found in 16% of patients as compared to 5% in the non-autoimmune individuals. Study by Irfan Yavasogl *et al*., 2009 reports that thyroid function and anti-thyroid antibodies should be performed as part of biochemical and immunological profile in RA patients. Jorge Cárdenas Roldán *et al*., 2012 in his study on the prevalence and impact of autoimmune thyroid disease (AITD) in patients with rheumatoid arthritis has reported that AITD is not uncommon in RA and should be systematically assessed since it is a risk factor for developing diabetes and cardiovascular disease. The study was extended to taking gender into consideration, female subjects showed higher prevalence of around 88% and male subjects around 12%, while in controls positivity was only in female patients. Similar to several previous reports the prevalence was higher in female compared to male even in controls consisting of non autoimmune individuals positivity was observed only in females. Presence of either anti TPO or anti -TG antibodies thus can trigger the apprearance of other autoantibodies in this case anti-CCP thus raising an alarm for autoimmune individuals with the risk for developing systemic autoimmune diseases like Rheumatoid arthritis.

## CONCLUSION

Higher prevalence of anti CCP antibodies in autoimmune subclinical hypothyroid patients compared to controls comprising non autoimmune hypothyroid patients clearly indicates that such patients are vulnerable for systemic autoimmune disease like Rheumatoid arthritis and hence should be screened for better prognosis. Nevertheless, more large-cohort prospective studies are needed in order to further establish the use of these antibodies in routine serological testing for diagnosed subclinical hypothyroid patients as well as healthy or high-risk individuals.

## References


Alberti L, Proverbio MC, Costagliola S, *et al*: Germline mutations of TSH receptor gene as cause of nonautoimmune subclinical hypo-thyroidism. J Clin Endocrinol Metab 2002; 87: 2549–2555.

Ambika, Gopalakrishnan, Unnikrishnan, Sanjay Kalra, RakeshKumar, Sahay, Ganapathi Bantwal, Mathew John, Neeraj Tewari, Prevalence of hypothyroidism in adults: An epidemiological study in eight cities of India, 17, 4, 2013, 647-52.

Anti-thyroid antibodies and thyroid dysfunction in rheumatoid arthritis: Prevalence and clinical value 2008, Vol. 41, No. 1, Pages 111-115. Fabiola Atzeni, Andrea Doria, Anna Ghirardello, Maurizio Turiel, Alberto Batticciotto, Mario Carrabba, and Piercarlo Sarzi Puttini.

Arasini B, Massarotti M. Rheumatoid arthritis and thyroid disease. Clin Exp Rheumatol. 2004; 22:265.

Autoimmune Thyroid Disease in Rheumatoid Arthritis: A Global Perspective Jorge Cárdenas Roldán, Jenny Amaya-Amaya,*et al*. Arthritis Volume 2012. Banal F, Dougados M, Combescure C, Gossec L: Sensitivity and specificity of the American College of Rheumatology 1987 criteria for the diagnosis of rheumatoid arthritis according to disease duration: a systemic literature review and meta-analysis. Ann Rheum Dis 2009 July;68:1184-1191

Canaris GJ, Manowitz NR, Mayor G, Ridgway EC: The Colorado thyroid disease prevalence study. Arch Intern Med 2000; 160: 526–534.

Chan ATY, Al Saffar Z, Bucknall RC. Thyroid disease in systemic lupus erythematosus and rheumatoid arthritis. Rheumatology 2001; 40: 353-354.

Delamere J, Scott D, Felix-Davies D. Thyroid dysfunction and rheumatic diseases. JR Soc Med 1982; 75:102–106.

Dessein PH, Joffe BI, Stanwix AE. Subclinical hypothyroidism is associated with insulin resistance in rheumatoid arthritis. Thyroid. 2004; 14: 443-446.

Dołęga B,Szechiński J.[Evaluation of thyroid gland function in patients with rheumatoid arthritis]. Reumatologia. 1996; 24:616 -618.

Dorner T, *et al*. Rheumatoid factor revisited. Curr Opin Rheumatol 2004; 16:246–53.

Gabriel SE. The epidemiology of rheumatoid arthritis. Rheum Dis Clin North Am 27 (2001) 269-281.

Irfan Yavasoglu, Taskin Senturk, Adil Coskun, and Zahit Bolaman. Rheumatoid Arthritis and Anti-Thyroid Antibodies. Autoimmunity. 2009 Feb; 42(2): 168–169.

Matsui T, Shimada K, Ozawa N, *et al*. Diagnostic utility of anti-cyclic citrullinated peptide antibodies for very early rheumatoid arthritis. J Rheumatol 2006; 33:2390-7

Niewold T.B., M.J. Harrison and S.A. Paget Anti-CCP antibody testing as a diagnostic and prognostic tool in rheumatoid arthritis .Q J Med 2007; 100:193–201

Peter J Charles, Darren Plant, Muslima Chowdhury,Jane Worthington, Patrick Venables Antibodies to thyroglobulin and thyroid peroxidase in rheumatoid arthritis: environmental and genetic associations .Ann Rheum Dis 2011;70(Suppl 2):A1–A94.

Prevalence of thyroid diseases and antithyroid antibodies in women with rheumatoid arthritis.

Przygodzka M, Filipowicz-Sosnowska A.Pol Arch Med Wewn. 2009 Jan-Feb;39-43.

Porkodi R, Ramesh S, Mahesh A, Kanakarani P, Rukmangathrajan S, Panchapakesa C. Thyroid dysfunction in systemic lupus erythematosus and rheumatoid arthritis. J Indian Rheumatol Assoc 2004; 12:0–0.

Schellekens GA, *et al*. The diagnostic properties of rheumatoid arthritis antibodies recognizing a cyclic citrullinated peptide. *Arthritis Rheum*. 2000:43:155-163.

Surks MI, Ortiz E, Daniels GH, *et al*: Subclinical thyroid disease: Scientific review and guidelines for diagnosis and management. JAMA 2004; 29: 228–238.

Vanderpump MP, Tunbridge WM, French JM, *et al*: The incidence of thyroid disorders in the community: a twenty-year follow-up of the Whickham Survey. Clin Endocrinol 1995; 43: 55–68.

Vincent C, *et al*. Anti-perinuclear factor compared with the so-called “antikeratin” antibodies and antibodies to human epidermis filaggrin, in the diagnosis of arthritides. *Ann Rheum Dis*. 1999; 58:42-48.

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