DOI: 10.7860/JCDR/2022/52706.16354

Original Article

Biochemistry Section

Clinical Association of TSH Receptor Antibody and Related Autoimmune Markers with Subclinical Graves' Disease: A Study from Eastern Indian Population

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ABSTRACT

Introduction: Graves' Disease (GD), the leading cause of hyperthyroidism is caused by interaction between various autoantibodies generated as a result of autoimmune dysregulation in genetically suspected individuals.

Aim: To study the prevalence of serum levels of Thyroid Stimulating Hormone (TSH) Receptor Antibody (TRAb), Antithyroid Peroxidase Antibody (Anti TPOAb) and Antithyroglobulin Antibodies (Anti TgAb) in subclinical cases of GD.

Materials and Methods: This was a hospital based cross-sectional study conducted in the Department of Biochemistry, in a tertiary care hospital, over a period of one and half years from January 2019 to June 2020. A total of 120 patients with subtle symptoms of GD of age group between 30 to 60 years and same number of age and sex matched healthy controls were enrolled for the study. Collection of data was done and serum TRAb was estimated by Enzyme Linked Immunosorbent Assay (ELISA), while AntiTPO and AntiTg antibodies were measured using Electrochemiluminescence Immunoassay (ECLIA).

Results: Serum levels of all the three antibody titres were found to be significantly higher in cases compared to controls. When diagnosis and differentiation of GD was done between cases and controls based on TRAb positivity (80.0%) or negativity (20.0%), it was found to have a sensitivity of 80% and specificity 98.3%. TRAb also had positive and negative predictive values of 97.9% and 84.3%, respectively. TRAb positive cases also had higher levels of Anti TPO and Anti TgAb in comparison to TRAb intermediate or negative group.

Conclusion: Prevalence of high titres of autoantibodies especially TRAb are pathognomonic of early GD. Their detection in covert stages of disease shall provide insight into predicting outcome and frame management strategies in patients with this thyroid disorder.

Keywords: Autoimmunity, Hyperthyroidism, Thyroid antibodies, Thyroid stimulating hormone

INTRODUCTION

Graves' disease, also known as toxic diffuse goitre, is an autoimmune disease that affects the thyroid [1]. It is the most common cause of hyperthyroidism (60-80%) apart from toxic multinodular goitre and toxic adenomas [2,3]. The exact cause is unclear; however, it is believed to involve a combination of genetic and environmental factors. Multiple nucleotide polymorphisms in Human Leukocyte Antigen-DR isotype (HLA-DR), immunoregulatory Cytotoxic T Lymphocyte-associated Protein-4 (CTLA-4), CD25, Protein Tyrosine Phosphatase Non receptor type 22 (PTPN22), FCRL3, CD226 genes as well as Thyroid Stimulating Hormone Receptor (TSHR) have been implicated to the development of GD [4].

The primary character of autoimmune thyroid disease is presence in serum of antibodies against Thyroid Peroxidase (TPO), Thyroglobulin (Tg) and the TSHR. T-cell-mediated autoimmunity is also demonstrated against the three primary thyroid antigens which on exposure to thyroid antigens or to similar peptide sequences elaborate various cytokines. Circulating autoantibodies specific to GD are directed against the TSHR and are mostly stimulatory in nature [5]. These antibodies compete against the natural TSH hormone for binding to its cell membrane receptors in different organs. Downstream mechanism including adenylate cyclase mediated multiple signaling pathways are now activated. Subsequently infiltration of extraocular muscles by activated T-cells occurs. There is release of cascade of cytokines like Interferon-γ (IFN-γ), Tumour Necrosis Factor (TNF), and Interleukin-1 (IL-1). In eye, this results in fibroblast activation, accumulation of glycosaminoglycans within the eye muscles that osmotically trap water causing muscle swelling [6].

Very little data are available from India, especially from eastern part with regard to the prevalence of autoimmune thyroid markers in general population especially for TRAb. Moreover, the correlation between TRAb titre level in GD cases in relation to severity of complications has not been assessed. Although both GD and Hashimoto's thyroiditis belong to different pathogenic mechanism, most of the contributing factors are similar for all autoimmune thyroid disorders. Immunogenicity of various autoantigens seem to vary depending on the state of presentation of clinical signs and symptoms and degree of severity. Hence, this study has been designed to explore the prevalence and titres of thyroid autoimmunity such as TRAb, Anti TPOAb, Anti TgAb in early clinical stages of GD in comparison to normal control population visiting a tertiary care hospital in Kolkata, West Bengal, India.

MATERIALS AND METHODS

The present study was a cross-sectional hospital-based study conducted in the Department of Biochemistry and Department of Endocrinology and Metabolism in a tertiary care hospital in Kolkata, West Bengal, India. A total of 120 clinically suspected patients of GD between 30 to 60 years of age, attending the Outpatient Department (OPD) and same number of age and sex matched healthy controls were selected for the study. Collection of data was done over a period of one and half years from January 2019 to June 2020. The study principles and procedures adhered to the ethical standards formulated in the Helsinki Declaration (1975, revised in 1983) and study was approved by the Institutional Ethics Committee (IPGME&R/IEC/2019/063, dated 04.02.2019).

Inclusion criteria: Patients with mild features of thyrotoxicosis attributable to GD like mild thyroid gland enlargement, eye signs with serum TSH level below and Free T4 (FT4), Free T3 (FT3) levels in upper level of normal reference range for individual parameter were included in the study.

Exclusion criteria: Patients treated for GD or symptoms, biochemical features of GD of more than three months duration and autoimmune thyroiditis cases with clinical and biochemical features of frank thyrotoxicosis were excluded from the study.

Serum obtained via centrifugation from clotted blood samples were analysed for routine thyroid function test parameters like FT4, FT3 and TSH using a solid-phase, enzyme-labeled chemiluminescent competitive immunoassay (IMMULITE 1000, Siemens, ADVIA Centaur, Siemens). Estimation of TRAb was done by Immunoenzymometric assay (Enzyme Linked Immunosorbent Assay Kit, RSR Limited, UK) [7]. Anti TPOAb levels were measured using solid phase enzyme linked chemiluminescent immunoassay while Anti TgAb were assayed using competitive immunoassay using direct chemiluminescent technology (IMMULITE 1000, ADVIA Centaur, Siemens) [8,9].

STATISTICAL ANALYSIS

Statistical Package for Social Sciences (SPSS) version 20 (IBM) statistical software was used for data analysis. All values were expressed as mean±Standard Deviation (SD). Continuous variables between cases of GD and normal population were compared using Student's t-test. Analysis of variance (ANOVA) was performed for comparing titre levels of autoimmune markers in cases. Probability value p-value <0.05 was considered to be statistically significant at a confidence limit of 95.0%.

RESULTS

Comparative results between levels of various autoimmune markers in cases and controls were significantly tilted in favour of cases of GD compared to controls (p-value <0.05; [Table/Fig-1]). In cases, there was significant association between titres of all three autoantibodies (p-value <0.05; [Table/Fig-2]). Among the cases (n=120), 96 were positive for presence of TRAb (>1.5 u/L). Two cases were in the intermediate group (TRAb value between 1.1 to 1.5 u/L). A total of 22 cases were negative for the presence of TRAb (value ≤1.0 u/L).

Variables		Cases	Controls	p-value
Age (years) (mean±SD)		41.28±6.7	40.83±4.9	0.5532
Sex	Male (n)	50	50	1**
	Female (n)	70	70	
FT4 (ng/dL) (mean±SD)		4.98±2.7	1.37±0.24	0.0001
TSH (µIU/mL) (mean±SD)		0.4±0.79	2.82±0.81	0.0394
TRAb (u/L) (mean±SD)		7.47±11.2	0.56±0.27	0.0001
Anti TPOAb (IU/mL) (mean±SD)		441.61±436.21	28.12±12.75	0.0001
Anti TgAb (IU/mL) (mean±SD)		146.06±325.85	28.93±10.04	0.0001

[Table/Fig-1]: Comparison between demographic features and laboratory parameters of patients with Graves' disease and controls.
*p-value <0.05: Statistically significant
**chi-square test

Variables	F-ratio value	p-value
TRAb (u/L)	63.91	0.0001
Anti TPOAb (IU/mL)		
Anti TgAb (IU/mL)		

[Table/Fig-2]: Analysis of variance (ANOVA) for levels of autoimmune markers in cases.
*p-value <0.05: Statistically significant

In cases, who were positive for TRAb (n=96), Anti TPOAb and Anti TgAb levels were higher than the upper limit of normal reference range in 95.8% and 82.3% cases, respectively. In comparison, among TRAb negative cases only 74.5% and 55.7% had high values of Anti TPOAb and Anti TgAb titres, respectively [Table/Fig-3]. In 92 cases presenting with high Anti TPOAb levels, 76 had high AntiTg Ab titres. In reverse, all cases with high Anti TgAb had high levels of Anti TPOAb. In controls (n=120), two cases were TRAb positive and 118 were TRAb negative. One TRAb positive control had higher Anti TPOAb (>40 IU/mL) level. Anti TgAb were within normal limits in both the TRAb positive controls. Overall, 26 controls were positive for Anti TPOAb (> 35 IU/mL) but had normal Anti TgAb levels. Anti TgAb was higher in 13 controls (>40 IU/mL) but all had normal Anti TPOAb level. In 79 controls there was no evidence of presence of significant titres of any the three antithyroid antibodies.

Variables	TRAb Positive (n=96)	TRAb Indeterminant/ Negative (n=24)	p-value
Female: Male Ratio	1.13	2.0	0.357
Anti TPOAb positive	95.8%	74.5%	0.039
Anti TgAb positive	82.3%	55.7%	0.026

[Table/Fig-3]: Comparison of Anti TPOAb and Anti TgAb prevalence in Graves' disease patients based on TRAb status.
*p-value <0.05: Statistically significant

When diagnosis and differentiation of GD was done between subclinical cases and controls based on TRAb positivity or negativity, it yielded a sensitivity of 80.0% and specificity 98.3%. TRAb also had positive and negative predictive values of 97.9% and 84.3%, respectively.

DISCUSSION

The objective of this study was to elucidate the appearance of TRAb, Anti TPOAb and Anti TgAbs in serum along with concomitant biochemical changes in thyroid hormone status in subclinical GD. In GD features of hyperthyroidism prevails over a backdrop of certain degree of chronic thyroiditis and which eventually culminates in thyroid hypofunction. Conversely, thyrotoxicosis may sometimes appear as the initial clinical symptom in patients with pre-existing Hashimoto's thyroiditis.

The present study population had cases of GD with high percentage of TRAb positivity (80.0%). Among these TRAb positive cases, 96.0% had high titres of Anti TPOAb and 82.0% had high Anti TgAb. This prevalence of high autoimmune markers in cases was indicative of the underlying autoimmune mechanism that was prevalent in the thyroid milieu and responsible for the thyroid dysfunction in cases. Whether TRAb negative patients could develop GD or not is an ambiguous issue.The present study had a minor count of two TRAb negative cases.

This being a rarity, clinical features, thyroid function test abnormalities, other related autoimmune antibody profile and imaging modalities are used in combination to arrive at a conclusive diagnosis in such cases [10]. The thyroid gland itself harbours B-cells that acts as the site for intrathyroidal autoantibody secretion in autoimmune thyroid disease. In mice suffering from Severe Combined Immunodeficiency (SCID) and having a deficiency of both T and B-cell lymphocytes, transplantation of Graves thyroid tissue, results in the evolution and detection in serum of various human thyroid autoantibodies, including TRAb. However, following management of GD by thyroidectomy and radioiodine, some patients continue to secrete autoantibody as evidence for antibody production from extrathyroidal sources [11]. Our study results had shown a positive association between the levels of all three autoimmune markers. This indicates that this underlying pathogenesis is unique for the development of all autoimmune thyroid disorders and is implicated for gradual development of clinical symptoms. GD represents only a part of the spectrum which presents itself in diverse forms from euthyroid state to subclinical entities and ultimately overt disease.

Thyroid hormone receptors belong to a subfamily of G-Protein-Coupled seven-transmembrane Receptors (GPCR). In GD, binding

of circulating TRAb autoantibodies causes activation of TSHR and the adenylate cyclaselinked signalling cascades. This plays a pivotal role in controlling thyrocyte growth and accentuated thyroid hormone secretion [12]. In patients of GD, TRAb are primarily stimulatory in nature. Other varieties of TRAb are also existent, like a TSH receptor blocking antibody and a neutral form of antibody with no effect on the receptor [13].

So far, with the existent diagnostic modalities TRAb is not detectable in normal population. Hence, detection of significant TRAb titres in cases is pathognomonic of GD in presence or absence of florid symptoms. Experimental evidence exists of induction of Graves like hyperthyroidism in mice by injecting plasmid or intact eukaryotic cells expressing the TSHR [14]. Transplacental transfer of maternal TRAb can induce foetal hyperthyroidism. Higher the maternal TRAb concentrations, the higher the risk for the foetus to develop hyperthyroidism [15].

In the past decade, different high-affinity monoclonal TRAbs with agonist activity belonging to the immunoglobulin G1 subclass have been characterised. The hypervariable Fab region on the heavy chain binds to aromatic amino acid rich receptor causing stimulating effects on cyclic Adenosine Monophosphate (cAMP) production [16]. Detectable levels of TRAb only in patients with GD is testament that these autoantibodies demonstrate disease exclusivity. In contrast, high prevalence of Tg antibodies and TPO assays, a (80-100%) detectable TRAb with discernible thyroid-stimulating activity is seen in GD [17].

TRAb levels are decreased on remission of GD and on persistence they predict recurrence [18]. In keeping with this hypothesis highest titres of TRAb are noted in patients with the most severe orbitopathy. The level correlates directly with the severity of Graves orbitopathy. Quantitation of TRAb thus is a useful marker for diagnosis and assessing disease activity in GD. Moreover, serum TRAb estimation in pregnancy is useful to predict the likelihood of developing neonatal thyrotoxicosis. TRAb possesses prognostic relevance in treated GD patients especially in response to immunosuppressive treatment [19]. Unfortunately, in cases with low or negative titres, the utility of this test is questionable. In this minority section of GD who remain TRAb negative even with modern Thyroid binding assays. it is speculated that they have intrathyroidal TRAb production which does not spill over to circulation [20].

The bone of contention as far as the treatment of GD patients with antithyroid drugs is concerned is to determine the appropriate duration of treatment. Antithyroid therapy modulates the course of the underlying autoimmune process, but complete remission after withdrawal of treatment is likely to occur only if the disorder has entered an inactive phase. Transition from an active state of disease to dormant state is heralded by the decline in the levels of TRAb. This is only likely to occur after prolonged treatment of GD.

Multiple factors have been implicated in preventing recurrence. These include a natural transformation of stimulating TRAb to blocking antibodies, simultaneous progression of concomitant autoimmune thyroiditis and iodine deficiency. These confounding factors have made the predictive value of TRAb measurement in GD recurrence extremely contentious. The situation is further complexed by non persistent TRAb titres which makes prediction of clinical outcome in GD based on these values much more difficult.

Hence, convention warrants unabated treatment with antithyroid drug for about 6 to 12 months. Tapering or drug withdrawal is indicated only when TRAb disappear and serum TSH level become normal. Our study results unveil the high level of autoimmune mechanism in GD as evident by the high level of TRAb titres in majority of the subclinical cases. This was further evident as significant percentage of cases had elevated antiTPO level prior at the onset of thyroid dysfunction in comparison to the controls. Our set of results showed that significant difference existed between cases and controls in terms of anti TPOAb level in a greater study population in comparison to the combined assessment of antiTPO and anti TgAb (96% vs 75%). Hence, no significant advantage was achieved from estimation of dual in cases. Anti TPOAb thus only can be used as a discrete marker. In reverse, when the merit of anti TgAb positivity was assessed alone and compared to the combined antiTPO and anti TgAb, lesser section of cases had isolated anti TgAb elevation. These observations clearly verified that anti TgAb is not a favourable parameter and should always be combined with anti TPO.

A two-year assessment conducted in San Carlos USA, for appearance of thyroid autoantibodies had shown that 68.6% of study subjects had high anti TPOAb values prior to development of subclinical hyperthyroidism. In 14.4% of cases synchronicity was observed between rise in anti TPOAb levels and onset of clinical features of hyperthyroidism. In 2 cases (1.7%) anti TPOAb titres had risen following the onset of subclinical or overt hyperthyroidism. However, 18 (15.3%) had normal anti TPOAb titre [21]. The results show good similar congruence with findings of Hutfless SM et al., where the pre-existent high antiTPO (57.0%) and antiTg (47.0%) autoantibodies were observed prior to the diagnosis of GD [22].

Parameters to predict relapse would help customise treatment to specific patient types in GD. However, as evident from previous study, Anti TPOAb was not sensitive in this regard [23]. In an epidemiological community survey conducted in Cochin, subclinical and overt hyperthyroidism was present in 1.6% and 1.3% of subjects [24]. Among the detected hyperthyroid cases one third had positive anti TPOAb levels and of them 39.0% had discernible goitre. High percentage of autoantibodies in cases and their prevalence in normal population as well, indicates the preponderance to develop autoimmune thyroid disorders in genetically susceptible individuals in India. In this study, high level of sensitivity and specificity (80.0% and 98.3%, respectively) of TRAb has been shown to pose good predictive value to diagnose and differentiate GD from normal population in early stages of disease.

Limitation(s)

A major limitation of the study was the limited population enrolled for the work. Multicentric studies are further required in this regard to correlate titre of various antibodies with disease severity and its early detection in covert stage. Nevertheless, the study is one of its kind in the context of paucity of studies conducted so far to determine the prevalence of TRAb in subclinical GD and normal euthyroid population.

CONCLUSION(S)

Prevalence of high titres of autoantibodies especially TRAb are pathognomonic of early GD. Their detection in covert stages of disease shall provide insight into predicting outcome and frame management strategies in patients with this specific thyroid disorder. A common understanding of molecular interplay in GD is that TRAb is known to enhance expression of thyroid antigens. In GD, breakdown of immune tolerance and subsequent thyroid infiltration by activated T-cell are caused not as a result of autoantibody-induced insult. There exists in GD a contemporaneous state of autoimmune thyroiditis leading to formation autoantibodies against TPO and Tg as well. The assessment of autoimmune markers especially TRAb in covert stages of GD shall help clinicians predict outcome and frame management strategies in patients with this disorder.

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PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Oct 05, 2021
- Manual Googling: Dec 14, 2021
- iThenticate Software: Jan 03, 2022 (6%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Oct 04, 2021**
Date of Peer Review: **Nov 06, 2021**
Date of Acceptance: **Jan 04, 2022**
Date of Publishing: **May 01, 2022**