SERUM ANTI-THYROID PEROXIDASE ANTIBODIES IN PATIENTS WITH ENDOGENOUS DEPRESSION

JAHANGIR SULTAN, AKIF QASIM, M. TAYYIB, ALLAH DITTA AND M. FAROOQ
Department of Pathology, Jinnah Hospital, Lahore

ABSTRACT
A total of 80 subjects were divided into two groups. Groups A included 20 normal healthy control subjects and group B included 60 subjects with endogenous depression Anti-TPO antibodies were performed by commercially available kits. Results were analysed by using students T test and level of significance was done. Anti-TPO antibodies were significantly raised in patients with endogenous depression.

INTRODUCTION

A depressive disorder is an illness that involves the body mood and thoughts in any given 1 year period and 9.5 percent of population suffer from depressive illness.[1] Depressive illness often interferes with normal functioning, cause pain and suffering to those who care about them. Severe depression can destroy family life as well as the life of the ill person.[2] Depression is often treated as a chronic condition due to frequent relapses that patient endures. The fact highlights the need for adequate treatment duration to prevent a quick relapse of the disorder but also a good follow up and disease awareness is needed to assist in the early education of future episode.[3]

Predisposing factors include family tendency to depression genetic, viral, neurological, neurodevelopmental, biochemical, psychological abnormalities and structure changes in brain. Depressive disorder affects approximately 18.8 million. American adult each year. One in four women and one in 10 men have a serious episode of depression during their life time. Children can also develop depression, which increase the risk of abuse problems and tendency for suicide. Depression is one of the leading causes of disability world – wide.[4-6]

World Health Organization (WHO) has predicted that by 2020, depression will be second leading cause of disability in the world after coronary heart disease.[5] It has been reported that physical changes in the body can be accompanied by mental changes as well. Medical illness such as stroke, heart attack, cancer, parkinson's disease and hormonal disorder can cause endogenous depression making the sick person apathetic and unwilling to care for his physical need, this prolongs the recovery period. In addition the faeces difficulties as financial problems, and/or stressful changes in life patterns can trigger depressive episode. Combination of psychological and environmental factors is involved in the cascade of depressive disorder.[7]

Thyroid Peroxides (TPO) antibodies: TPO is the key thyroid enzyme catalysing both the iodination and coupling reaction for the synthesis of thyroid hormone. It is membrane bound and found in the cytoplasm and in high concentration on the apical surface of thyrocytes having microvilli. Its molecular weight is between 100 and 105 – Kda and previously it was known as thyroid microsomal antigen B. Multiple T&B Cell epitopes exists within the molecule and the antibody response to TPO is restricted to the level of germ line heavy and light chain variable (V) region.[9] Anti-TPO auto-antibodies are found in over 90% of patients with autoimmune hypothyroidism and Graves' disease. Together with TG antibodies these are the predominant antibodies in AH. Anti-TPO antibodies are mainly of the IgG class with IgG_1 and IgG_4 subclasses in excess.[10]

Thyroid antibodies play a role in the pathogenesis of depression. It is seen that higher level of serum thyroid peroxidise antibodies has been shown in 10% women and 2% of men are vulnerable to develop depression.[11]

The pituitary gland compensates by producing more TSH, as a result of which thyroid pumps out more hormone. In the early stages of this process, T_3 and T_4 levels are normal but TSH is elevated. If the illness progresses, thyroid hormone level falls and TSH rises dramatically.[12]

The present study is planned to perform thyroid function test and thyroid peroxidase antibodies in patients with endogenous depression. By monitoring the levels of TSH, T_3, T_4 and anti-TPO antibodies of, impending thyroid related disorders can be predicted.

This study will be beneficial to patients of endogenous depression who are not responding to anti depressant drugs. The patients with endogenous depression due to hypothyroidism do not respond to anti depressant drugs until they receive thyroid hormone therapy. The present study will be of much

help in patients with endogenous depression and fill the gap in our country in this field methodology. Eighty subjects were included in this study and were divided in to two groups.
Group A: Included 20 normal healthy control subjects which are twenty.
Group B: Included 60 patients with endogenous depression.

Between Jan. and Dec. 2011, 60 newly diagnosed depressive patients in the age range of 18 – 65 years, having first episode of illness reporting at OPD *Punjab Institute of Mental Health* and diagnosed as per DCR (Diagnostic Criteria for Research) of ICD – 10 (WHO, 1992) were selected. Twenty healthy controls were taken from dermatological clinic. A careful history, through physical examination and relevant laboratory investigations were performed to rule at any evidence of endocrinological, hepatic, renal, cardiac or other chronic systemic illness. Significant alcoholism or other substance abuse and pregnancy or oral contraceptive use (in case of female subjects). The depressive patients were rated on Hamilton's depressive rating scale (Hamilton, 1967) to assess the severity of depression. The controls were matched with the depressive patients in respect to age, sex and socio economic status. Five ml of blood was collected and serum was separated. The results were analysed by using student's t test and level of significance was done.[13]

Principle of the Test
Highly purified human thyroid peroxidase (TPO) is bound to microwells. Antibodies against this antigen, if present in diluted serum or plasma, bind to the respective antigen. Washing of the microwells removes unspecific serum and plasma components. Horseradish peroxidase (HRP) conjugated anti-human IgG immunologically detects the bound patient antibodies forming a conjugate / antibody / antigen complex. Washing of the microwells removes unbound conjugate. An enzyme substrate in the presence of bound conjugate hydrolyzes to form a blue color. The addition of an acid stops the reaction forming a yellow end – product. The intensity of this yellow color is measured photometrically at 450 nm. The amount of colour is directly proportional to the concentration of IgG antibodies present in the original sample.

Contents of the Kit
Package size 96 determ
Qty. 1 Divisible microplate consisting of 12 modules of 8 wells each, coated with highly purified human thyroid peroxidase (TPO). Ready to use. 6 vials, 1.5 mL each combined Calibrators with IgG class Anti-TPO antibodies (A – F) in a serum / buffer matrix (PBS, BSA, NaN_3 < 0.1% (w/w)) containing: IgG: 0; 33; 100; 330; 1000; 3000 IU/mL. Ready to use 2 vials, 1.5 mL each Anti-TPO Controls in a serum / buffer matrix (PBS, BSA, NaN_3 < 0.1% (w/w)) positive (1) and negative (2), for the respective concentrations see the enclosed QC insert.

Ready to use
1 vial, 20 ml sample buffer (Tris, NaN_3 < 0.1% (w/w)), yellow, concentrate (5 ×).

1 vial, 15 mL Enzyme conjugate solution (PBS, Proclin 300 < 0.5% (v/v)), (light red) containing polyclonal rabbit anti-human IgG, labelled with horseradish peroxidase. Ready to use.

1 vial, 15 mL TMB substrate solution. Ready to use 1 vial, 15 mL Stop solution (1 M hydrochloric acid). Ready to use 1 vial, 20 mL Wash solution (PBS, NaN_3 < 0.1% (w/w)), concentrate (50 ×).

Specimen Collection, Storage and Handling
1. Five ml of blood was collected and serum was separated. Collected whole blood specimens using acceptable medical techniques to avoid haemolysis.
2. Allow blood to clot and separate the serum by centrifugation.
3. Test serum should be clear and non-haemolysed. Contamination by haemolysis or lipaemia is best avoided, but does not interfere with this assay.
4. Specimens may be refrigerated at 2 – 8°C for up to five days or stored at -20°C up to six months.
5. Avoid repeated freezing and thawing of serum samples. This may result in variable loss of autoantibody activity.
6. Testing of heat – inactivated sera is not recommended.

Test Procedure
1. Prepare a sufficient number of microplate modules to accommodate controls and prediluted patient samples.
2. Pipet 100 µL of calibrators, controls and prediluted patient samples in duplicate into the wells.
3. Incubate for 30 minutes at room temperature (20 – 28°C).
4. Discard the contents of the microwells and wash 3 times with 300 µL of wash solution.
5. Dispense 100 µL of Enzyme conjugate into each well.
6. Incubate for 15 minutes at room temperature.
7. Discard the contents of the microwells and wash 3 times with 300 µL of wash solution.
8. Dispense 100 µL of TMB Substrate solution into each well.
9. Incubate for 15 minutes at room temperature.
10. Add 100 µL of stop solution to each well of the

modules and incubate for 5 minutes at room temperature.

11. Read the optical density at 450 nm and calculate the results.

Bi-chromatic measurement with a reference at 600 – 690 nm is recommended.

The developed colour is stable for at least 30 minutes. Read optical densities during this time.

RESULTS

Results and level of significance of different groups are given in table.

Table 1: *Anti-TPO antibodies in Group A (control) and Group B (Patients with endogenous depression.*

Test Group (A) (Control) Group (B)
(Subjects with endogenous depression)
A Vs B
Anti-TPO antibodies (IU/ml) 6.62 ± 2.58
23.17 ± 42.08
P < 0.01
(HS)

DISCUSSION

Serum anti-TPO antibodies

In this study, serum anti-TPO antibodies in patients of endogenous depression Group (B) were elevated when comparing with control group (A) and difference was highly significant statistically ($p < 0.01$). There is increased prevalence of anti thyroid antibodies in patients with endogenous depression. In these patients, thyroid autoimmunity may be weakly associated with depression. It may be due to psychiatric effects of autoimmune thyroiditis which in increased level of anti-TPO in depressed patients. High levels of these anti-TPO antibodies can inhibit thyroid functions. Depression often coexist with autoimmune thyroiditis, suggests that depression may cause alteration in the immune system or that in fact, it is an autoimmune disorder itself. This high level of antibodies indicate that autoimmune process is active.[14] The findings are compatible with those of Null (2000),[11] Goldman et al (1998),[15] Nemeroff (1998)[16] and Carta et al (2000)[17] who also observed elevated anti-TPO antibodies in patients with endogenous depression. Kousae et al (1998)[18] studied in pregnant women that anti-TPO antibodies in 14 (17.5%) cases.

Significantly high prevalence of anti-TPO antibodies were seen during pregnancy ($p < 0.001$).

Conclusions

The study suggests that individuals in the community with thyroid autoimmunity may be at high risk for endogenous depression and anxiety disorders. The psychiatric disorders and the autoimmune reaction seem to be rooted in the same (and not easy correctable) aberrancy in the immuno-endocrine system. Should our results be confirmed, the findings may be of great interest for future preventive and case finding projects. A systematic screening for mood disorders in anti-TPO + subjects and a systematic evaluation for thyroid diseases and thyroid autoimmunity in subjects with mood disorders may be advisable.

ACKNOWLEDGEMENT

We are grateful to the administration of Jinnah Hospital for allowing to do this work.

REFERENCES

1. The Family Intervention Guide to Mental: Recognizing and Getting Treatment (Bodie Morey and Kim T. Muesser, 2007.
2. Mary Jan England and Lesli J. Sim, Editors; Committee on Depression, Parenting Practices, and the Healthy Development; National Research Council; Institute of Medicine 2009.
3. Annabel Prce, Lauren Ratan, Ewa Okon – Pocha, Alison Evans Koravangattu Valsraj, J HIGGINSON, Mattew Hotop J Neurosurg Psychiatry 2011; 82: 914-923. Published on line First: 10 May 2011 do:1136/tnnp.2010.23862
4. Belmaker RH, Agam G. Major depressive disorder. N Eng J Med. 2008 JAN 3: 55-68.
5. Hetrick S, Merry S, McKenzie J, Sindal P, Proctor M. Selective Serotonin reuptake inhibitors (SSRIs) for depressive disorders in children and adolescents. Cochrane Database Syst Rev. 2007 Jul 18 (3): CD004851
6. Bridge JA, Iyengar S, Sslsry et al. Clinical response and risk for reported suicidal ideation attempts antidepressant treatment: a meta-analysis of randomized controlled trials. JAMA. 2007 Apr 18: 1683-95.
7. Bastiaens L. Response to Antidepressant Treatment in a Community Mental Center. Community Mental Health Journal, December 2004; Vol. 40, No. 6: P. 561-567.
8. McLachian, S.M. Rapport B. The molecular biology of thyroid peroxidase: Cloning, expression and role as auto-antigen in autoimmune thyroid disease. Endo. Rev. 1992; 13: 192-206.
9. McIntosh, R, Waston, P, Weetman A. Somatic hypermutation in autoimmune thyroid disease. Immunol. Rev. 1998; 162: 219-231.
10. Silva, L.M., Chavej J., Canalli, M.H., Zanetti C.R. Determination of IgG sub-classes and avidity of antithyroid per-oxidase antibodies in patients with subclinical hypothyroidism – a comparison with patients with over thypothyroidism. Horm Res. 2003; 118-24.
11. Joffe R. T. Antithyroid antibodies in major depress 23 AUG 2000. DOI: 10.1111/ j.1600-0447.1987.tb02925.×
12. Johnsons AM, Eagles JM. Increased prevalence of thyroid antibodies and risk factors in 4 clinical hypothyroidism. Br. J. Psychiatric 1999; 175: 336-9.

13. Bland M. An introduction to medical statistics. 1st ed Oxford University Press 1998: 165-87.
14. Null G. The Food – MOODING – Body concentration. Seven stories press, New York, NY. 2000: 85-92.
15. Goldman LS, Wise TN, Brody DS (Eds). Psychiatry for primary care physician. American Medical Association 1998; 289: 82-89.
16. Nemeroff CB. Clinical significance of psychoneuroendocrinology in psychiatric focus on the thyroid and adrenal. J. Clin. Psychiatry 1989: 13-20.
17. Carta MG, Lovisell A, Hardoy MC, Meassa S, Cadeddu M, Sardu C. et al. The link between thyroid autoimmunity with anxiety and depression in community. BMC psychiatry 4: 25.
18. Kousar N, Tayyab M, Allah Ditta, Abid SM, Chaudary NA. Clinical importance of thyroid peroxidase antibodies (TPO – AB) in pregnancy. Pakistan J Med Res. 1922: 137 (1).