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RESEARCH ARTICLE

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LECTIN BASED HEMAGGLUTINATION ASSAY: A SCREENING TEST FOR THYROID DISORDERS

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ABSTRACT

The Present study demonstrates the diagnostic characteristics of Pedilanthus tithymoloides, agglutinin (PTA), lectin based hemagglutination method for screening of thyroid disorders as compared with RIA as gold standard. The result estimates 90% sensitivity, 95.55% specificity and 92.63% observed agreement with RIA test in thyroid disorders. The result of hyperthyroidism, hypothyroidism and cretinism are discussed and these results project Lectin based hemagglutination as simple, reliable, cost effective and an alternative method of choice for screening of thyroid disorders.

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KEYWORDS

Lectin, Hemagglutination, Glycoprotein

INTRODUCTION

Thyroid disorder is one of the major health problems, which continues to seriously damage precious human resources.[1] It leads to much wider spectrum of disorders beginning with intra-uterine life and extending through childhood to adult hood.[2]

The older methods for evaluation of thyroid status of an individual by measurement of the basal metabolic rate has proved unwieldy, unreliable and imprecise at many instances.[3] Currently physicians rely largely on indirect measures such as thyroid hormone level in blood and clinical evaluation to ascertain the thyroid hormone status of a person. Although these measures are often sufficient on a more clinical level, but they turn out to be expensive in bulk screening. Hence there is a need for a simple, reliable and low-cost method, which can evaluate the thyroid hormone status of an individual during mass screening.

Carbohydrate moieties of glycoproteins and glycolipids are distributed on the outer surface of the cell membrane. They interact with antibodies, enzymes, hormones, lectins, viruses and other molecules.[4] They have an enormous potential for encoding biological information but precise role played by these biomolecule are yet to be understood. Lectins divalent or monovalent, carbohydrate binding proteins having defined specificity, reacts with such surface glycoconjugate. Scientific data supports that cell surface glycoconjugates get altered during disease state,[5,6,7] and it is expected that each such alterations should have a characteristic pattern of lectin binding [8]

Due to their specificity towards glycoconjugates Lectins have proved to be powerful tool for study of glycosylation changes occurring in many diseases.[9, 10] Lectin based methods are simple, less expensive and have comparable specificity. Hence the present study has been conducted to evaluate the efficacy of lectin based hemagglutination method and possibilities to use it as an alternative method of screening in thyroid disorders.

MATERIAL AND METHODS

One hundred and ninety individuals comprised the study population, out of which one hundred subjects were known, diagnosed cases of thyroid disorders. Remaining ninety healthy individuals formed normal control group.

T3, T4 and TSH hormone assay were conducted with commercially available Radio Immune Assay (RIA) kit.

Pedilanthus tithymoloides agglutinin (PTA) purified in our lab, 0.07 mg/0.1ml protein contents having specificity for Galactose > N-Ac-Galactosamine, was used as reagent to find hemagglutination titer of PBS washed 2%(v/v) RBC suspension.

1.8 ml blood samples were collected in 0.2ml of 3.8% Na-citrate as anticoagulant. The blood was centrifuged to separate erythrocyte from plasma. The erythrocytes were washed thrice in 0.05M Phosphate Buffered Saline (PBS) of pH 7.2 and 2% (v/v) erythrocyte suspension was prepared for the hemagglutination titer assay. Assay was carried out in microtiter plate by the procedure of Rudiger.[11]

Results obtained from commercially available Radio Immune Assay (RIA) kit for T3, T4 and TSH hormone assay were used as Gold Standard for comparing and evaluating the result of hemagglutination titers obtained by *Pedilanthus tithymoloides, agglutinin* (PTA)

having specificity towards Galactose > N- Ac-Galactosamine.

Hemagglutination test were performed thrice on each 2 % (v/v) PBS washed normal, healthy control RBC samples. *Pedilanthus tithymoloides, agglutinin* (PTA) having 0.07 mg/0.1ml protein gave 41 – 65 units as cut off range, for hemagglutination titer. The results were interpreted after comparing with the hemagglutination titer with cut off range. Samples having titer value above cut off range were marked as hyperthyroid and below as hypothyroid. Differentiation of hypothyroid and cretinism was based on age factor.

RESULTS AND DISCUSSION

Result obtained from one hundred and ninety blood samples analysed for T3, T4 and TSH hormone levels by RIA, showed, 100 samples positive for thyroid disorders, where as ninety samples remained negative by RIA suggesting normal group.

Samples analyzed by RIA when subjected for hemagglutination titer (HT) test using PTA, revealed 90 out of 100 thyroid disorder cases were detected positive. Where as 90 out of 90 normal samples turned out normal.

Table-1 indicates 90% sensitivity and 95.55% specificity, of hemagglutination titer value obtained by PTA. It also shows 90% positive predictive value and 92.63% observed agreement between RIA and PTA-HAT method in various thyroid disorders. Interestingly As indicated in table, the hemagglutination test titer results remain undaunted even when they were categorized in to Hypothyroid, Hyperthyroid and Cretinism groups after comparing with RIA.

To summarize our finding, though higher sensitivity and specificity of RIA method has made it a preferred method, but looking at the simplicity and cost effective factor, hemagglutination titer method has proved equally good as it has comparable specificity and sensitivity. Thus PTA-HAT method may become a method of choice in screening test where just normal or impaired thyroid status is important. In near future such lectin based methods may find a place as routine diagnostic reagents. Also it needs our effort to prepare it for the quantitative assay.

Table No: 1
Diagnostic characteristics of lectin based hemagglutination method using PTA Lectin having 41 to 65 units as normal cut off titer value at 0.07 mg/0.1ml protein concentration compared against RIA Kit method

Diagnostic Characteristic	Thyroid disorders Total	Under specific condition		
		Hyperthyroid	Hypothyroid	Cretinism
Sensitivity	90%	92.63%	87.5%	82.97%
Specificity	95.55%	96.77%	97.4%	97.085%
Positive predictive value	90%	94.62%	95.45%	92.85%
Negative predictive value	89.58%	96.77%	92.59%	92.59%
False positive	4.44%	5.37%	4.55%	7.14%
False negative	10%	7.36%	12.5%	17.02%
Likelihood ratio of positive test	20.25	28.76	33.65	29.64
Likelihood ratio of negative test	0.15	0.076	0.128	0.175
% agreement with RIA	92.63	95.2	93.6	92.66

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