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RESEARCH ARTICLE

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ALTERED EXPRESSION OF PEDILANTHUS TITHYMALOIDUS LECTIN BINDING GLYCOPROTEIN ON ERYTHROCYTES IN THYROID DISORDERS, A PRELIMINARY REPORT

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ABSTRACT

Measurable morphological changes in plasma and cell membrane glycoprotein are noted in various diseases. Lectin mediated agglutination of red blood cell can reveal such alteration. Present investigation was undertaken to assess the binding characteristics of *P. tithymaloidus agglutinin* on erythrocytes from thyroid disorders to find any alteration in membrane glycoproteins. In our study, hyperthyroidism has shown significantly increased hemagglutination titer (82.3 ± 52.9), where as decreased hemagglutination titer has been observed in hypothyroidism (34.1 ± 23.1). Observed finding substantiate that thyroid induced metabolic stress bring change in membrane glycoproteins which is reflected in the form of altered lectin binding activity on erythrocyte.

KEYWORDS

Lectin, Hemagglutinin, Erythrocyte, Thyroid

INTRODUCTION

Morphological changes are noted on cell surface, including erythrocytes, under metabolic stress which lead to permanent lesion on cell surface [1,2,3]. Measurable changes in glycosylation of plasma or cell membrane proteins are observed in disease state [4,5,6,7]. Lectin, Sugar binding proteins or glycoproteins of non immune origin can detect such changes. Lectin mediated agglutination of red blood cell is credited to the interaction of the lectin with glycoproteins which can reveal alteration arising in glycoprotein on cell surface under metabolic changes or pathogenesis.

Thyroid hormone action is typically biphasic in character. Increase or decrease activity of various enzymes observed in erythrocytes points towards complex and multidirectional effect of thyroid hormone on red cell [8]. Hence, there is a considerable reason to expect characteristic glycoprotein changes on the surface of erythrocyte from thyroid disorders, where cells are exposed to two extreme points of metabolic condition. The present investigation was undertaken to assess the biding characteristics of Galactose and Galactosamine specific *Pedilanthus tithymaloidus* agglutinin (PTA) on erythrocytes of thyroid disorders.

MATERIALS AND METHODS

The subjects included in this study were randomly selected from suspected thyroid disorder cases, which were referred to from the Department of ENT, Medicine and Endocrinology of our Hospital, for various investigations to Department of Biochemistry.

Seventy eight individuals (17 Males and 61 Females) were between 16-63 years age group with the mean age group of 28.2 ± 11.6 years comprised the study population. Thyroid status of the individuals was evaluated by estimation of T3, T4 and TSH using ELISA Kit method. On the basis of TSH level and Kleeg G et al criteria patients were categorized into hypothyroid and hyperthyroid groups [9]. Known cases of thyroid disorders but presently maintaining Euthyroid status were excluded from the study group.

Ninety six age and sex match healthy individuals having no disease, particularly thyroid disorders, formed control group.

Blood samples were obtained by anticubital venous puncture and transferred to 3.8% citrate solution. Red blood cells were transferred into 0.05 M phosphate buffer (pH 7.2) containing 0.15 M sodium chloride (PBS) and repeatedly washed till supernatant was clear. A 2% suspension of RBC was prepared in PBS and used for hemagglutination study.

EXTRACTION AND PURIFICATION OF LECTIN FROM P. tithymaloidus (PTA):

About 10 ml of *P. tithymaloidus* latex was directly collected into about 90 ml of 0.05 M phosphate buffer, pH 7.2 containing 0.15 M sodium chloride (PBS), by making an incision of 0.5 to 1.0 mm deep on the stem with a clean knife. Diluted latex was subjected to repeated freezing and thawing. The latex clot was separated from remaining extract by centrifugation at 3000rpm for 30 minutes and was washed with PBS to remove the last traces of soluble proteins. Supernatant including PBS washings were centrifuged at 3000rpm for 30 minutes. The resulting clear supernatant was acidified to pH 5.0 with 0.1 M acetic acid. The supernatant was then brought to pH 8.0 with 0.1 M sodium hydroxide. Any precipitate formed was discarded after centrifugation. Next supernatant was subjected to 80% acetone

fractionation. The precipitate obtained was dissolved in about 10 ml of PBS and further purified on sephadex column. PTA was shown to be Galactose and N-acetyl Galactosamine specific when inhibition of agglutination using different sugars was determined as described by Premratna et al 1981[10]. Also PTA was found to be non specific for blood group.

Protein Estimation:

Protein content was estimated by the method of Lowery et al 1951[11].

Hemagglutination assay:

The agglutinability of erythrocyte with PTA was assayed by serial dilution using microtiter plate by the procedure of Rudiger 1983[12].

The highest dilution of lectin causing 100% agglutination was regarded as the titer. This was then converted to the base of one mg of protein. Statistical analysis was done by students 't'

RESULT AND DISCUSSION

Table No. 1

	Number	Age group Years	Mean ± SD	Male	Female
Thyroid Cases	78	16 – 63	28.2 ± 11.6	17	61
Control	96	20 - 60	31.4 ± 8.2	35	61

52.68% thyroid disorder was observed in young age i.e. 21 – 40 years age. Sexwise females were three and half times more susceptible for thyroid disorders as compared to males.

Peripheral blood erythrocytes of healthy controls were tested at least 3 times with PTA lectin for its hemagglutination test. Similar hem agglutination titers were observed on repetition, which suggested that the glycosylation of normal peripheral blood erythrocyte membrane proteins had receptors for the employed PTA lectin with which they reacted quantitatively and they were sufficiently stable to consider pathological alterations. Though large range of variation in titer was observed due to serial dilution method, still patients having thyroid dysfunction had shown statistically significant difference in mean hem-agglutination titer.

Erythrocytes from hypothyroid individual have shown significant decrease in mean hemagglutination titer ($P<0.01$) for Galactose and N acetyl galactosamine specific PTA when compared to normal healthy erythrocyte hemagglutination titers.

On the other hand, significantly, increase in mean hemagglutination titer was observed in hyperthyroid state for Galactose and N acetyl galactosamine specific PTA. ($P< 0.001$)

Table No. 2

	No of cases= n	Male	Female	TSH µunit/ml	PTA hemagglutination titer
Control	96	35	61	5.9 ± 1.28	52.7±17.4
Hypothyroid	30	9	21	34.7 ± 4.1	34±23.1**
Hyperthyroid	48	8	40	3.68 ± 1.42	82.3±52.9*

P< 0.001, ** P<0.01

Increased hemagglutination titer was observed in hyperthyroidism concord with the findings of increase sialilation of epithelial cell in autoimmune thyroiditis[13], masking of Galactose epitopes in mandibular cell of hypothyroid rat.[14] and pointing towards increased sialilation in masking of Galactose epitopes.

Decreased hemagglutination titer was observed in hypothyroidism correlates well with negative influence of thyroid hormone on red cell membrane protein[15,16]. This might be the result of either exposure of extra Galactose epitopes by shading off of terminal Sialic acid or extra incorporation of Galactose epitopes in the cell membrane.

Normal erythrocyte was recognized by presence of Sialic acid as terminal molecule and galactosyl as the penultimate sugar in membrane oligosaccharides[17]. Micro environment of the cell was constantly influenced by metabolic reactions occurring in the cell. A metabolic disorder brought changes in the microenvironment of the cell[1,2,3]. Observed changes in erythrocyte membrane lectin receptors were pointing towards change in the glycoprotein on the erythrocyte surface. This seemed to be participating in the adaptive processes, to tolerate metabolically altered environment in varied level of thyroid hormones. Cell and its membrane could not tolerate such variation in environment where they survived. As a result, macromolecules of the cell membrane had undergone adaptive changes to maintain equilibrium in order to protect the cell against altered environment, but these adaptive changes in the cell membrane which some times could lead to diseases.[4,5,6,7]. Thus, the result of present study adds further weight to the evidence that membrane specific alteration do occur in metabolically stressed erythrocyte in thyroid hormone related disorders. Further, evaluation of serum and membrane Sialic acid, glycoprotein and glycolipid content of erythrocyte membrane may put some focus on the binding pattern of the membrane glycoprotein.

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