

## CLINICAL APPLICATION OF SERUM LEVELS OF GLYCOPROTEIN IN BRONCHIAL ASTHMATIC PATIENTS

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### ABSTRACT

Asthma is a chronic inflammatory disease of the airways associated with marked activation of the immune system, leading to increased production of various biochemical mediators. The serum glycoproteins are involved in various inflammatory disorders. Therefore the aim of present study is to investigate the significance of glycoproteins in asthma, serum levels of protein bound hexose, hexosamine, and fucose were measured by spectrophotometric methods in 20 healthy subjects, 20 asthmatic patients not undergoing therapy and 30 asthmatic patients undergoing therapy. Serum glycoprotein levels in the asthmatic patients not undergoing therapy were significantly higher than those in the healthy subjects and those in the asthmatic patients undergoing therapy ( $p < 0.01$  and  $p < 0.01$ , respectively). Serum glycoprotein levels in the asthmatic patients were significantly different from those patients under treatment. The possibility that a factor release in to serum from injured or necrotic tissue during inflammation might be responsible for stimulation of glycoprotein synthesis. The study suggested that the glycoproteins evaluated may be useful in diagnosis and treatment monitoring of asthmatic patients.

**Keywords:** Bronchial asthma, Inflammatory disorders, Serum Glycoproteins.

### INTRODUCTION

Glycoproteins are macromolecular protein linked with carbohydrate found in the cell surface, which forms the principal component of animal cells. Protein bound hexose, hexosamine, and fucose are the basic components of the glycoproteins. They play an important role in membrane transport, cell differentiation and recognition, the adhesion of macromolecules to the cell surface, and the secretion and absorption of macromolecules. They are also found as enzymes, hormones, blood group substance and components of cellular and extracellular membranes<sup>1</sup>.

The level of different types of serum glycoproteins are maintained within a narrow range in healthy subjects<sup>2</sup> but is elevated in many pathological conditions viz. autoimmune disease<sup>3</sup>, cardiovascular disease<sup>4,5</sup>, diabetes mellitus<sup>6</sup>, cancer of cervix, uterus and breasts<sup>7-9</sup>, trauma<sup>10</sup>, prolonged bed rest<sup>11</sup> and arthritis<sup>12</sup> including psychiatric disorders<sup>13</sup>. The causes leading to changes in its level in serum under various pathophysiological conditions are not fully understood. Among the several conditions in which the level of glycoprotein in serum is increased are inflammatory disorders<sup>14,15</sup>. Bronchial asthma a complex chronic airway disorder characterized by airway inflammation, mucus hypersecretion, elevated serum IgE level, and airway hyperresponsiveness<sup>16,17</sup>. Since bronchial asthma is inflammatory disease it was thought of interest to determine serum protein bound hexose, protein bound hexosamine, and protein bound fucose in same disease. Hence, the present study was undertaken to study the changes in the level of serum glycoproteins as protein bound hexose, protein bound hexosamine, protein bound fucose, in patients of asthma with and without treatment and to investigate the effect of treatment on the levels of serum glycoprotein in patients of asthma to correlate with clinical improvement using normal subjects.

### SUBJECTS AND METHODS:

This study was carried out in total 70 subjects comprising both the normal subjects and patients. The study groups comprised 20 normal subjects (14 male and 6 females) with age ranges from 25-55 years, 20 asthmatic patients without treatment (12 male and 8 females) with age ranges from 24-55 years. 30 asthmatic patients undergoing treatment (13 male and 17 female) with age ranges from 25-75 years. After ethical clearance, the study was conducted on patients visited to Out Patient Department (OPD) of S.S.G. Hospital, Baroda.

The diagnosis of patients of asthma was performed by standard clinical criteria by consultant physician. Healthy normal subjects were selected on the basis of good health as evidenced by the medical history, complete physical examination and routine

laboratory tests performed prior to the commencement of the study. They met the inclusion and exclusion criteria. All the subjects were instructed to abstain alcoholic products throughout the study period. None of the subject and patient had any organ dysfunction. Healthy normal subjects and patients did not receive any medication during four weeks prior to the commencement of the study. They were instructed during treatment not to take any over the counter (OTC) medications subsequently until the completion of the study. Informed consent was obtained from healthy normal subjects and legal guardian of the patients of asthma. Then patient were given a treatment (prednisolone, salbutamole, aminophylline) for one month.

Blood samples were collected from normal subjects and patients. After collection, the blood samples were centrifuged to separate serum. All the serum samples were stored at -20°C until analysis. Biochemical analysis was performed on serum samples for estimation of protein bound hexose, protein bound hexosamine and protein bound fucose by well established methods. All the reagents were of analytical reagent grade. All the analytical procedures were standardized for reproducible and feasible results.

#### Protein bound hexose:

Protein bound hexose was estimated by the method of Weiner and Moshin<sup>12</sup>. In this method, the hexose moiety of glycoprotein conjugates precipitated by ethanol at room temperature is determined by orcinol reaction at 540 nm.

#### Protein bound hexosamine:

Protein bound hexosamine was estimated by the method of Winzler<sup>18</sup>. In this method serum proteins are precipitated by ethanol and hexosamines are liberated from the glycoproteins by acid hydrolysis. Acetylation in alkaline medium cyclizes the hexosamine to pyrrole derivative that couple with paradimethyl amino bezaldehyde forming color complex, which was determined photometrically at 530 nm.

#### Protein bound fucose:

Dische and Shettles<sup>19</sup> have described methods for estimation of protein bound fucose, which lead themselves to the determination of methyl pentose in serum because methyl pentose and fucose gave identical optical densities under the condition of the determination. However, since only fucose has been demonstrated in the serum glycoproteins or in related mucoid. It is reasonable to report methyl pentose value as fucose. Values are determined by measurement of optical density at wavelength 396 nm and 430 nm.

### Statistical analysis

Serum concentrations of glycoproteins are expressed as mg%. Data were expressed as mean±SD. Statistical significance was analyzed using student's t test. P values < 0.01 were considered statistically significant.

### RESULTS AND DISCUSSION

Table 1 summarizes the results of measurements of the serum concentrations of protein bound hexosamine, protein bound hexose and protein bound fucose in normal subjects, in patients of bronchial asthma without treatment and in patients of bronchial asthma undergoing treatment.

**Table 1: Protein bound hexose, hexosamine and fucose in serum samples from normal subjects, patients with asthma with or without treatment**

GROUPS	PROTEIN BOUND HEXOSE (mg %) Mean±SD (Range)	PROTEIN BOUND HEXOSAMINE (mg %) Mean±SD (Range)	PROTEIN BOUND FUCOSE (mg %) Mean±SD (Range)
Normal Subject	118 ± 12.7 (95-138)	82.05±9.3 (66-110)	8.3±2.9 (4-14)
Asthmatic patients without treatment	197.5± 21.7* (160-247)	107.03±18.3* (71-143)	16.53± 4.0* (9-27)
Asthmatic patients undergoing treatment	157.0±27.5* <sup>§</sup> (145-199)	77.3±12.5* <sup>§</sup> (72-100)	11.55±3.6* <sup>§</sup> (9-16)

Values are given as Mean ± SD. (p< 0.01).

\*Values are statistically significant as compared with Normal Subjects

§Values are statistically significant as compared with asthmatic patients without treatment

Results show the significant increase of the protein bound sugars in the patients with treatment and without treatment as compared to healthy subjects. A statistically significant decrease is observed in glycoprotein level after treatment in bronchial asthmatic patient but the levels of serum glycoprotein did not reach to the values of serum glycoprotein of control subjects. On the other hand, the serum glycoprotein levels were almost identical in both sexes and were not influenced by age in normal healthy subjects and patients both.

Current data suggest that an increased concentration of serum glycoprotein in patients with asthma with or without treatment reflects that glycosylation is enhanced in bronchial Asthma.

According to Jamieson<sup>14</sup> inflammation results in an increase in the levels of variety of glycoproteins in serum and it was also found that serum glycoprotein increased in various immunopathological conditions<sup>20</sup>. Bronchial asthma is immunological and inflammatory condition and also there was an increase in immunoglobulin level was observed in blood and these immunoglobulin forms a set of glycoproteins. According to Masuda<sup>21</sup> the glycoprotein level was higher in sputum from allergic asthma. So it was thought of interest to study serum glycoprotein levels of bronchial asthma patients.

The levels of glycoproteins (hexose, hexosamine and fucose) in serum of normal subjects is in agreement with those found in previous study<sup>18,22</sup>. This study demonstrates that the significantly higher levels of glycoprotein were observed in the serum of the asthmatic patients when compared with the normal subjects. Elevation of this fraction has been reported in numerous experimental animal models<sup>23-25</sup>.

By a variety of experimental approaches it has been established that the liver is the principal site of serum glycoprotein synthesis<sup>23,26-28</sup>. Previous studies have reported that, in rats showing acute inflammatory reaction is the source of glycoprotein and that appearance of this protein in serum is a result of de novo synthesis<sup>29</sup>.

However, other mechanism such as local release of glycoprotein into blood stream subsequent to degradation of connective tissue or a stimulation of glycoprotein synthesis secondary to process of cellular proliferation can not be excluded as contributing factor depending upon the particular glycoprotein fraction under consideration.

Several lines of investigation have demonstrated that an apparent stimulation of general and specific glycoprotein synthesis occurs during injury or inflammation<sup>29-31</sup>. The possibility that fraction release into serum from injured and necrotic tissue might be responsible for stimulation of glycoprotein synthesis<sup>29</sup>.

The significant decrease was observed in glycoprotein level after treatment with prednisolone, salbutamole, aminophylline in bronchial asthmatic patient shows that the production of glycoprotein was as a consequence of inflammation; where as reduction of inflammation was a sequel to healing of inflammation. There is also an agreement amongst the authors that the occurrence of this substance after injury forms part of a homeostatic control system, which with treatment assists to curtail the progress of certain inflammatory reactions<sup>32</sup>. The study suggested that the glycoprotein evaluated may be useful in diagnosis and treatment monitoring because treatment also shows significant decrease in glycoprotein level of asthmatic patients.

In conclusion, it is suggested that the observed alteration in serum glycoprotein reflect a complex interrelationship involving inflammatory reaction, degradation of connective tissue, process of cellular proliferation, injury. On the basis of present study it can be concluded that serum glycoprotein levels may serve as an indicator of bronchial asthma. Although serum glycoprotein levels appears to be a nonspecific indicator of asthma. Moreover fall in level of serum glycoproteins can also be used as an indicator of efficacy of treatment as well as clinical improvement.

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