STUDY OF CHANGES IN SERUM GLYCOPROTEIN LEVELS IN OSTEOPOROSIS PATIENTS

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ABSTRACT

Glycoproteins can be simply defined as proteins which have carbohydrates covalently attached to their peptide portion. Glycoproteins are proteins which contain monosaccharides like hexose, hexosamine, fucose and sialic acid. This study was carried out on total 110 subjects, comprising the normal subjects and the patients of osteoporosis. Normal subjects and all the patients of osteoporosis were diagnosed and selected by orthopedic surgeon from S.S.G. Hospital, Baroda and then taken for study. Blood (5 ml) was collected in plain bulbs and serum is separated by centrifugation. Serum is kept under refrigeration at 4ºc until use. There is an increase in Serum glycoproteins level in Patients of Osteoporosis (With Postmenopausal Women) and in Patients of Osteoporosis (Other than Postmenopausal Women). Also there is decrease in Serum glycoproteins levels observed in Patients Undergoing Treatment. But Treatment did not bring down the level of Serum glycoproteins to normal level. So it is suggested that the increase in Protein Bound Hexose (PBH), Protein Bound Hexosamine (PBHA), Protein Bound Fucose (PBF), Protein Bound Sialic Acid (PBSA) and Protein Bound Carbohydrates (PBC) in Patients of Osteoporosis could be due to enhanced glycosylation of Protein in Osteoporosis. Also Osteoporosis usually causes no symptoms until a fracture occurs but it can cause back pain or loss of height. so evaluation of serum PBH, PBHA, PBF, PBSA and Carbohydrates (PBC) may be used as an alternative methods and markers for diagnosis of Osteoporosis.

KEYWORDS: Glycoproteins, hexose, hexosamine, fucose, sialic acid. Osteoporosis.
INTRODUCTION
Glycoproteins can be simply defined as proteins which have carbohydrates covalently attached to their peptide portion. Glycoproteins are proteins which contain monosaccharides like hexose, hexosamine, fucose and sialic acid. There are obviously appreciable quantities of all the components in serum. The protein bound hexose (PBH), protein bound haxosamine (PBHA), protein bound fucose (PBF) and protein bound sialic acid (PBSA). Hence the amount of protein bound carbohydrates in serum is a direct measure of serum glycoproteins levels.

The glycoproteins as a group have multiple and complex function. They are found as enzymes, hormones, blood group substances, cellular and extra cellular membranes, both soluble and insoluble components of connective tissue. So increase or decrease in glycoproteins level may affect physiological functions of different organ systems of human body. Many investigators have demonstrated that the concentration of the glycoproteins in human serum is abnormally high in a number of physiological and pathological condition.[3,4,10]

It is mentioned that increased activity of parathyroid gland may enhances the release of glycoproteins from the bone.[11] Since osteoporosis is the bone disease and no literature data is available for the serum glycoproteins levels in osteoporosis, it was thought of interest to study the changes in serum glycoproteins level in osteoporosis.

MATERIALS AND METHODS
This study was carried out on total 110 subjects, comprising the normal subjects and the patients. Normal subjects and all the patients of osteoporosis were diagnosed and selected by orthopedic surgeon from S.S.G. Hospital, vadodara and then taken for study.

Blood (5 ml) was collected in plain bulbs and serum is separated by centrifugation. Serum is kept under refrigeration at 4ºc until use. The patients and normal subjects were divided into four groups as shown below

Table 1. Numbers of groups for collecting data of Serum PBH, PBHA, PBF, PBSA levels.

<table>
<thead>
<tr>
<th>No.</th>
<th>Group</th>
<th>Total no.</th>
<th>Male</th>
<th>Female</th>
<th>Range of Age (Year)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal subjects</td>
<td>40</td>
<td>18</td>
<td>22</td>
<td>18-70</td>
</tr>
<tr>
<td>2</td>
<td>Patients of osteoporosis (with)</td>
<td>40</td>
<td>-</td>
<td>40</td>
<td>50-80</td>
</tr>
</tbody>
</table>
The blood samples from all the subjects were analyzed for Protein bound Hexose (PBH), Protein bound Hexosamine (PBHA), Protein bound Fucose (PBF), Protein bound Sialic Acid (PBSA).

All the methods selected for the present study are standard procedures, giving reproducible results and these methods were feasible to carry out in our laboratory. All chemicals were of analytical grade.

**Protein Bound Hexose (PBH)**

Serum protein bound hexose is estimated by the method of Weimer and Moshin.[16]

**Procedure**

1) To the 0.1 ml. of serum in a centrifuge tube, add 5 ml of ethanol and mix.
2) Centrifuge it for 15 min., decant, suspend the precipitate in 5 ml of 95% ethanol and then centrifuge and decant.
3) Dissolve the precipitated proteins in 1 ml. of 0.1 N NaOH.
4) To the 1 ml. of aliquot, 1 ml. of blank (H₂O) and 1 ml. of standard, add 8.5 ml of orcinol-H₂SO₄ reagent(Alembic Pharma.) and mix.
5) Cap the tubes with glass marble to minimize evaporation and place in a water bath at 80°C for exactly 15 minutes.
6) Cool the tubes in the tap water and take reading at 540 nm. The amount of PBH is then calculated in terms of mg %.

**Protein Bound Hexosamine (PBHA)**

The protein bound hexosamine is estimated by the method of Rimington.[5]

**Procedure**

1) To 0.1 ml of serum in test tube add 5 ml of 95% ethanol and mix.
2) Centrifuge for 15 minutes, decant, suspend the precipitate in 5 ml 95% ethanol and centrifuge and decant.
3) To precipitated proteins add 2 ml of 3 N HCl and hydrolyze in a boiling water bath with an air condenser for 4 hours.
4) Neutralize the hydrolysate with 3 N NaOH until it is barely alkaline to litmus and dilute up to 10 ml.
5) To 1 ml aliquot, 1 ml of H2O for blank and 1 ml of glucosamine (Sun Pharma), add 1 ml of acetyl-acetone reagent and mix.
6) Cap the tubes with marbles to prevent evaporation and place in a boiling water bath for 15 minutes.
7) Cool the tubes in tap water, add 5 ml of 95% ethanol & mix.
8) Add 1 ml of Ehrlich’s reagent (Sun Pharma), mix well, dilute to 10 ml with 95% ethanol.
9) Take reading after 30 minutes at 530 nm.

**Protein Bound Fucose (PBF)**

Few quantitative determination of the protein bound fucose content of serum in normal individuals or in patients with pathological conditions has been carried out.

**Procedure**

1) To duplicate test tubes, add 0.1 ml serum and 5 ml of 95% ethanol and mix.
2) Centrifuge for 15 minutes, decant, suspend the precipitate in 5 ml of 95% ethanol, centrifuge and decant.
3) Dissolve the precipitated proteins in 1 ml of 0.1N NaOH.
4) To the tubes and to 1 ml of H2O for a blank and 1 ml of the methyl pentose standard (Cadila Pharma), add 4.5 ml of ice cold H2SO4-H2O mixture. Mix well while maintaining the solutions cold in ice bath.
5) Heat for exactly 3 minutes in a boiling water bath and cool in tap water.
6) Add 0.1 ml of the cystein reagent (Alembic Pharma.) and mix immediately (omit this regent from one of the serum samples to correct for non-specific color development).
7) After 60 to 90 minutes at room temperature take optical density reading at 396 nm and 430 nm with distilled water set at zero.

**Protein Bound Sialic Acid (PBSA)**

**Procedure**

1) Add 4.8 ml. of 5% TCA (Alembic Pharma.) slowly with shaking to 0.2 ml of serum and to 0.2 ml. of the orsomucoid standard (Sun Pharma) in 15 × 150 mm. test tubes.
2) Place the tubes in a boiling water bath for exactly 15 minutes with a glass marbles to prevent evaporation. Cool the tubes by immersion in water and filter.
3) Pipette 2 ml. of the clear filtrates into each of two 15 × 150 mm. test tubes.
4) Place 4 ml. of the DPA reagent into one of each pair of tubes and 4 ml. of the acid mixture containing no DPA (Sun Pharma) into the other.
5) Prepare reagent blank (2 ml. of 5% TCA plus 4 ml. of DPA reagent).
6) Mix, cap the tubes with a glass marble and immerse the tubes in a boiling water bath for exactly 30 minutes.
7) Cool the tubes in water and determine the optical density at 530 mμ with a reagent blank set at zero.

RESULT

Table 2 - Data of Changes in Serum PBH, PBHA, PBF, PBSA levels in various groups.

<table>
<thead>
<tr>
<th>No.</th>
<th>Groups</th>
<th>PBH mg %</th>
<th>PBHA mg %</th>
<th>PBF mg %</th>
<th>PBSA mg %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal Subjects</td>
<td>101.32-140.42</td>
<td>80.31-129.21</td>
<td>5.12-12.3</td>
<td>52.29-8.29</td>
</tr>
<tr>
<td></td>
<td>Range (mg %)</td>
<td>128.93 ± 8.46</td>
<td>103.34± 11.61</td>
<td>8.78±1.78</td>
<td>61.07± 4.61</td>
</tr>
<tr>
<td></td>
<td>Mean + SD (mg %)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Osteoporosis Patients (With Postmenopausal Women)</td>
<td>186.29-229.29</td>
<td>168.59-201.52</td>
<td>9.56-17.10</td>
<td>71.29-101.1</td>
</tr>
<tr>
<td></td>
<td>Range (mg %)</td>
<td>203 ± 10.85</td>
<td>182.44± 8.15</td>
<td>12.84± 1.98</td>
<td>84.51± 6.04</td>
</tr>
<tr>
<td></td>
<td>Mean + SD (mg %)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Osteoporosis Patients (Other than postmenopausal Women)</td>
<td>178.20-204.30</td>
<td>162.25-185.62</td>
<td>8.89-17.88</td>
<td>78.22-96.50</td>
</tr>
<tr>
<td></td>
<td>Range (mg %)</td>
<td>92.68± 7.66</td>
<td>76.02± 7.65</td>
<td>12.39± 2.46</td>
<td>86.33± 5.74</td>
</tr>
<tr>
<td></td>
<td>Mean + SD (mg %)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Osteoporosis Patients Undergoing treatment</td>
<td>166.29-183.29</td>
<td>141.30-161.25</td>
<td>9.75-16.71</td>
<td>69.15-85.55</td>
</tr>
<tr>
<td></td>
<td>Range (mg %)</td>
<td>175.82± 5.51</td>
<td>152.29± 5.34</td>
<td>12.52± 1.98</td>
<td>77.42± 4.90</td>
</tr>
</tbody>
</table>

PBH - Protein Bound Hexose.
PBHA - Protein Bound Hexosamine.
PBF - Protein Bound Fucose.
PBSA - Protein Bound Sialic Acid.

DISCUSSION

According to result, there is an increase in Serum glycoproteins level in Patients of Osteoporosis (With Postmenopausal Women) and in Patients of Osteoporosis (Other than...
Postmenopausal Women). Also there is a decrease in Serum glycoproteins levels observed in Patients Undergoing Treatment. But Treatment did not bring down the level of Serum glycoproteins to normal level.

The definite cause on why these increases in level of glycoproteins take place is not known but Catchpole H. (1950) has proposed that serum glycoproteins may arise as a result of depolymerisation of the ground substances of connective tissue with release of solubilized components into the circulation.

CONCLUSION
There is an increase in Serum glycoproteins level in Patients of Osteoporosis (With Postmenopausal Women) and in Patients of Osteoporosis (Other than Postmenopausal Women). Also there is a decrease in Serum glycoproteins levels observed in Patients Undergoing Treatment. But Treatment did not bring down the level of Serum glycoproteins to normal level.

So it is suggested that the increase in Protein Bound Hexose (PBH), Protein Bound Hexosamine (PBHA), Protein Bound Fucose (PBF), Protein Bound Sialic Acid (PBSA) and Protein Bound Carbohydrates (PBC) in Patients of Osteoporosis could be due to enhanced glycosylation of Protein in Osteoporosis. Also Osteoporosis usually causes no symptoms until a fracture occurs but it can cause back pain or loss of height. so evaluation of serum PBH, PBHA, PBF, PBSA and Carbohydrates (PBC) may be used as an alternative methods and markers for diagnosis of Osteoporosis.

ACKNOWLEDGEMENT
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REFERENCES