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CHANGES IN LEVELS OF SERUM GLYCOPROTEINS IN MAJOR DEPRESSIVE DISORDERS

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ABSTRACT

The present study deals with estimation of levels of fractions of serum glycoproteins, protein bound hexose (PBH), protein bound hexosamine (PBHex), protein bound fucose (PBF), protein bound sialic acid (PBS) and protein bound carbohydrate (PBC) in thirty patients of Major Depressive Disorders (MDD) in comparison with thirty normal subjects. In patients of MDD, the level of PBH, PBHex, PBF, PBS and PBC were significantly higher as compared to the normal subjects ($p<0.05$). In patients of MDD, after one-month treatment with fluoxetine, the levels of PBH, PBHex, PBF, PBS and PBC were significantly decreased as compared to the levels of these fractions in same patients of MDD before beginning of the treatment ($p<0.05$). Based on findings of the present study, it can be concluded that changes in the level of serum glycoproteins level before and after treatment with fluoxetine can be correlated with clinical status of MDD.

KEY WORDS

Major Depressive Disorders, Serum Glycoproteins.

INTRODUCTION

Glycoproteins can be simply defined as proteins that have carbohydrate moiety covalently attached to their peptide portion. The glycoproteins as a group have multiple and complex function and are found as enzymes, hormones, blood group substances and as constituents of extracellular membranes. These are organic compounds, composed of both a protein and carbohydrate monosaccharides, usually hexose, hexosamine, fucose and sialic acid, joined together covalently linked to polypeptide chain. The level of different types of serum glycoproteins are maintained within a narrow range in health (1), but is elevated in many pathological conditions viz. tuberculosis (2), autoimmune disease (3), cardiovascular disease (4, 5), diabetes mellitus (6), cancer of cervix, uterus and breasts (7, 8, 9), trauma (10), prolonged bed rest (11) and arthritis (11, 12) including psychiatric disorders (13,14).

In psychiatric diseases, major depressive disorders (MDD) is most common and it may range from a very mild condition, bordering on normality, to severe (psychotic) depression accompanied by hallucinations and delusion. According to Diagnostic and Statistical Manual of Mental Disorders-IV (DSM-IV), a major depressive disorder is defined as one or more major depressive episodes, without history of manic or mixed episodes. A major depressive episode must last at least 2 weeks; typically, a person is either depressed or losses interest in most activities. A person diagnosed with major depressive episode must also experience at least four symptoms from a list that includes changes in appetite and weight, changes in sleep and activity, lack of energy, feeling of guilt, problems in thinking and making decision and recurring thoughts of death or suicide. Since the glycoproteins vary in their relative content of serum of four monosaccharides, the net change in the total level would also vary, depending upon the monosaccharides used for determination (15,16). In psychiatric disorders such as schizophrenia, elevated levels of serum glycoprotein has been reported (17). So, it was thought of interest to investigate the changes in the level of serum glycoprotein in patients of MDD. Hence, the present study was undertaken to study the changes in the level of serum glycoproteins as protein bound hexose (PBH), protein bound hexosamine (PBHex), protein bound fucose (PBF), protein bound sialic acid (PBS) and protein bound carbohydrate (PBC) in patients of depression before and after one month fluoxetine treatment and to investigate the effect of fluoxetine treatment on the levels of serum glycoprotein in patients of depression to correlate with clinical improvement using normal

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subjects as control.

MATERIALS AND METHODS

The study was carried out on 60 subjects comprising 30 normal healthy volunteers (18 male and 12 female) and 30 patients of MDD (17 male and 13 female) with the range of 30 to 50 Years. After ethical clearance, the study was conducted on patients visited to Psychiatry Out Patient Department (OPD) of S.S.G. Hospital, Baroda. The diagnosis of patients of MDD was performed by standard clinical criteria, which was confirmed by using the structured clinical interview for DSM-IV Axis-1 disorders Patient Edition, administered by a clinical psychologist. 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 depression. Then patient of MDD were given fluoxetine (Prodep® 20 mg once a day; Sun Pharmaceuticals, Mumbai), an antidepressant drug for one month.

Sample analysis: Blood samples (3 ml each) were collected from normal subjects only once and from patients of depression at two occasions, before beginning of the treatment and after one month of the treatment. After collection, the blood samples were centrifuged to separate serum. All the serum samples were stored at -70°C until analysis. Biochemical analysis was performed on serum samples for estimation of protein bound hexose, protein bound hexosamine, protein bound sialic acid, protein bound fucose and protein bound carbohydrate (16,18). All the reagents were of analytical reagent (AR) 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 (12). 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 (16). 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 (19) have described methods for estimation of protein bound fucose, which lead themselves to the determination of methyl pentose in serum. The method involves heating the serum sample with sulfuric acid for 3-10 minutes followed by the addition of cysteine. Satisfactory specifically for methyl pentose has been achieved by determining the optical densities at two wavelengths in order to correct for color developed by other sugars. Application of the procedure to serum glycoproteins has further supported the conclusion that reaction substance is methyl pentose. Rhamnose and fucose gave identical optical densities under the condition of the determination and can be used as standard. However, since only fucose has been demonstrated in the serum glycoproteins or in related mucoid it appears reasonable to report methyl pentose value as fucose. Methyl pentose value is determined by measurement of optical density at wavelength 396 nm and 430 nm.

Protein bound sialic acid: Protein bound sialic acid or neuraminic acid was determined by the method of Seibert (20). In this method, a red brown colour is produced when serum is treated with tryptophan in the presence of strong perchloric acid. Absorption spectra was recorded at 500 nm.

The amount of PBH, PBHex, PBF, PBS and PBC were calculated in terms of milligram percentage.

Statistical analysis

Data were expressed as mean±SEM. The data so obtained was analyzed to obtain appropriate conclusions. Student 't' test was employed to find out the statistical significance. The levels of serum glycoproteins of normal healthy subjects were compared with that of patients of MDD before treatment and levels of serum glycoproteins in patients of MDD before treatment and after treatment were also compared.

RESULTS AND DISCUSSIONS

The results of the present study are presented in Table 1. The serum glycoprotein levels were almost identical in both sexes and were not influenced by age in normal healthy subjects and patients both. A statistically significant difference in mean concentrations of PBH, PBHex, PBF, PBS and PBC was observed between normal healthy subjects and patients of depression. An elevated level of serum glycoproteins PBH, PBHex, PBF, PBS and PBC was observed in patients of depression in comparison with

Table 1. Mean levels of serum glycoproteins PBH, PBHex, PBF, PBS and PBC in normal subjects and in patients of depression before and after treatment

Group	PBH mg%	PBHex mg%	PBF mg%	PBS mg%	PBC mg%
Normal subjects (N=30)	108.65±2.23	81.45±2.05	9.0±0.58	66.45±0.74	265.52±3.79
Patients of depression before treatment (N=30)	195.82±2.74*	100.53±1.15*	15.5±0.86*	159.56±1.84*	491. 30±3.94*
Patients of depression after treatment (N=30)	115.84±3.73**	89.88±1.48**	11.52±0.65**	140. 56±1.94**	410. 76± 4.96**

*p<0.05 (Normal subjects Vs patients of depression before treatment)

**p<0.05 (Patients of depression before treatment Vs patients of depression after treatment)

normal healthy subjects. A statistically significant fall in values of PBH, PBHex, PBF, PBS and PBC was found in patients of depression after one-month of treatment with fluoxetine in comparison with values of PBH, PBHex, PBF, PBS and PBC obtained before beginning of the treatment in the patients of depression. After one-month follow-up, a significant decrease in serum glycoprotein levels was observed with fluoxetine therapy, the levels of serum glycoproteins did not reach to the values of serum glycoprotein of control subjects.

This study demonstrates that the level of PBH, PBHex, PBF, PBS and PBC were elevated in patients of depression and the level of these glycoproteins returns slightly above the values of normal healthy subjects upon one month fluoxetine treatment. It was observed that one-month treatment with fluoxetine significantly reduces the serum glycoprotein levels as compared to patients of MDD before treatment. Lack of serotonin is often associated with depression. Restoring the normal or enhanced level of this neurotransmitter acts as mood enhancer. Fluoxetine is a mood-enhancing drug, which acts in the central nervous system by inhibiting the re-uptake mechanism of serotonin into the synapse. Since serotonin is not degraded in the synaptic cleft, fluoxetine promotes a prolonged presence of serotonin keeping the post-synaptic membrane active. Thus, It is suggested that the increase in serum levels of PBH, PBHex, PBF, PBS and PBC may be due to glycosylation of proteins, may be due to biosynthesis of glycoproteins in liver or due to release of preformed proteins in patients of depression. The mechanism of decrease in levels of glycoproteins in patient of depression after one-month treatment with fluoxetine is unclear, but is may be due to its regulating action on above mechanisms of glycoprotein release.

Kremer *et al.* (21) has demonstrated the elevated levels of alpha-1 acid glycoproteins in depression. In another study carried out by Varma *et al.* (13) in schizophrenics, in comparison with normal subjects, matched for age and sex both, the mean concentration of each of the protein bound carbohydrate component was significantly higher. The electrophoretic patterns for serum glycoprotein showed increases in alpha-2 and beta globulin in schizophrenics. The contents of glucose and arabinose were higher in serum glycoprotein obtained from psychiatric patients (17). Similarly in the present study elevated level of different type of serum glycoproteins was observed in patients of depression. The present study results support the previous study findings.

Thus, the present study clearly shows the diagnostic importance of serum glycoproteins. Although serum glycoprotein levels appears to be a nonspecific indicator of depression. On the basis of present study findings it can be concluded that serum glycoprotein levels may serve as an indicator of MDD. 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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