Increased Serum Sialic Acids in Depression.

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Summary:

**Background:** Total serum sialic acid (TSA) and lipid associated sialic acid (LSA) have not been measured yet in depressive disorders.

**Aim:** The present study was undertaken to show if there is any change in the concentration of different forms of sialic acids in patients with depressive disorder.

**Methods:** TSA and LSA levels have been estimated in serum of 72 patients with depression, in addition to 50 healthy controls.

**Results** reflect a significant increase (p<0.05) in the TSA and LSA in the depressed patients as compared with healthy controls.

**Conclusions:** It could be concluded that TSA and LSA could reflect an immune like response to depression associated with increase in the sialylation of different glycoproteins.

**Key Words:** Serum Total Sialic acid , Depression , Free Sialic acid , Bound Sialic acid , Lipid Associated Sialic acid.

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Introduction:

Depression is among the most common diagnosis assigned by psychiatrists in private practice [1]. The term depression has been used variously to describe an emotional state, a syndrome, and a group of specific disorder [2].

Katon and Sullivan (1990) [3] concluded that 6% of patients in primary care settings and 11% of medically in-patients have major depression, accompanied by a 3% incidence in general population. Major depression disease is associated with a high level of morbidity and mortality.

Despite intensive attempts to establish its etiologic or pathophysiological bases, the precise cause of major depressive disorder is not known. It is now well established that pain and depression are related [4-5]. Most common among these are endocrine abnormalities such as hypothyroidism [6-7], diabetes mellitus [8], Cushing's disease [9], cardiovascular diseases [10], CNS disorders such as cerebrovascular diseases [11] and Parkinson's disease.

The term sialic acids denotes a member of a family comprising more than 20 natural derivatives of neuraminic acid, an acid amino sugar in pyranose form with nine carbon atoms [12]. The preferred localization of sialic acids is the outer cell membrane where these sugars often occur in high concentration and are components of glycoproteins, gangliosides or polysaccharides. The electronegative charge of a human erythrocyte, for example, is mainly due to a dense coat of about 20 million sialic acid molecules [13].

Many substances were measured in depressed patients to evaluate their possible role in the etiology of depression. For instance different types of receptors [17-18], cytokines [19], serum electrolytes [20-21], hormones [22-23], cholesterol [24] and different trace elements [25-27].

The effect of sialic acids on the neurotransmission has not been studied yet. In this paper we tried to estimate the possible changes in the level of serum sialic acids in depression for the first time. The investigation in the (Internet and Pubmed) and papers published until August 2004 failed to find any previous study about the possible role of sialic acids in psychiatric disorders.

**Materials and Methods:**

**A-Patients:**

The study included 72 depressed patients aged (36.2 ± 14.4years) (mean ± standard deviation). The patients were diagnosed by the psychiatrists using a semi-structured psychiatric interview schedule for the diagnosis of depressive disorder based on the ICD-10. Patients were evaluated by full medical history to exclude any existing systemic disease that may affect the parameters to be diagnosed, particularly diabetes, liver disease, renal disease and chronic drug intake; otherwise the patient was excluded from the study. Consent was obtained from the patient or his close relatives.

**B-Controls:**

Fifty apparently healthy subjects were selected as a control group. Their sex and age were comparable to those of patients. None of these
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Subjects was obese, alcoholic, or having a history of heart diseases, other metabolic disorders, and none of the females was pregnant or on contraceptive pills.

C- Blood Samples:

Ten milliliters of venous blood samples were drawn by utilizing disposable needle and plastic syringes from each patient and control. The samples were transferred into clean plain tube. Haemolyzed samples were discarded. The blood was left at room temperature for 10 minutes for clotting, centrifuged at 3000 rpm for 10 minutes, and then serum was separated and divided into three parts.

Sialic acids:

1- Total Sialic Acid (TSA):

TSA was measured using Svennerholm method\(^2\) as modified by (George et al 1981)\(^2\):

**Resorcinol Reagent Preparation:**

Six hundred milligrams of resorcinol was dissolved in 60ml of 28% HCl, 40ml of water and 25 micromole of copper sulfate.

**Assay:**

In brief, 20µL of serum were diluted to 500µL in a test tube with distilled water. Then 0.1 ml of 0.04M periodic acid solution was added. The mixture was thoroughly mixed and allowed to stand in an ice bath for 20 minutes. After the addition of 1.25 ml of resorcinol reagent, the solution was mixed and placed in an ice for 5 minutes. The solution was then heated at 100°C for 15 minutes, cooled in tap water and 1.25 ml of tertiary butyl alcohol solution (95%) was added. After vigorous mixing, the tubes were kept in a 37°C water bath for 3 minutes, cooled at room temperature and the absorbance was measured at 630 nm. The concentration was obtained from the standard curve developed from different concentrations of n-acetyl neuraminic acid (NANA).

2- Lipid Associated Sialic Acid (LSA):

LSA was measured according to procedure of Katopodis and Stock (1980)\(^3\), in which 50µL of serum were extracted with 3ml of chloroform: methanol (2:1 v/v) at 4°C. The lipid extract was partitioned with 0.5 ml of cold distilled water and the aqueous layer containing LSA was precipitated with 50µL of phosphotungstic acid (1g/ml). After centrifugation, the supernatant was aspirated and the precipitate was re-suspended in 1ml of distilled water and one milliliter of resorcinol reagent was added to the tube, mixed and placed in boiling water for exactly 15 minutes. The tubes were transferred to an ice and water bath and left for 10 minutes. To the ice cold tube 2 ml of butyl acetate-(n-butanol) 85:15v/v mixture were added at room temperature, vortexed, and centrifuged for 5 minutes at 2500 rpm. The extracted blue color was read at 580 nm and the amount of lipid bound sialic acid is determined by use of the following formula:

\[
LSA \text{ mg/dL} = \frac{x \times 100000}{y \times 50 \times 1000}
\]

Where \(x\) = Concentration of NANA obtained from standard curve for the sample.

\(y\) = 1 ml. of supernatant: volume of entire supernatant.

3-Determination of Bound Sialic Acid (BSA):

Chromogen formed by periodate oxidation is destroyed at 37°C, whereas the chromogen obtained from the oxidation of the glycosidically bound sialic acid is stable at this temperature\(^2\). BSA was estimated using the protocol mentioned for TSA except that the oxidation step was carried out at 37°C not at 0°C.

4- Determination of Free Sialic Acid (FSA):

Free sialic acid was determined from the difference of the level indicated with the oxidation at 0°C and that obtained by oxidation at 37°C as demonstrated for TSA and BSA.

Results:

Figure (1) shows the serum concentration of different sialic acids in depressed patients in comparison with healthy controls. Total serum sialic acid (TSA) and bound serum sialic acid (BSA) were significantly increased in depressed patients. While the concentration of lipid associated sialic acid (LSA) and free sialic acid (FSA) in depressed patients showed no significant difference.

![Figure(1): Serum concentrations of total sialic acid (TSA), bound sialic acid (BSA), lipid associated sialic acid (LSA), and free sialic acid (FSA) in depressed and control groups.](http://www.simpopdf.com)
Discussion:

The increase in serum total sialic acid (TSA) in depressed patients is significant and this increase can be explained by the fact that many researchers showed a frequent immune response (31, 32) and increase in acute phase reactants in depressed patients (33-34). Because more than half of sialic acids in serum are derived from acute phase proteins (35), hence, the increase in acute phase proteins (that contain sialic acids) in response to stress or inflammation is the possible cause of increase TSA in depressed patients.

Sialic acids is a terminal component of the reducing end of carbohydrate chain of glycoproteins and glycolipids including hormones and enzymes present in serum and tissue (36) and 99% of sialic acids is bound to glycoproteins and glycolipids (35). Hence, the change in sialic acid concentration reflects a wide change in plasma glycoproteins, enzymes, and hormones. In addition, the increase in sialic acid is a consequence of sialylation of some proteins that influence many important processes such as platelet aggregation (37), clotting of fibrinogen (38), capillary permeability (39), uptake of lipoprotein into vessel walls (40), and in vivo aggregation of erythrocytes (41). These reports showed that increased serum sialic acid concentration was associated with atherosclerotic disease (42-43). This fact could indicate that, the depressed patients are at high risk for atherosclerotic disorders.

Raised serum sialic acid concentration have been shown to predict cardiovascular and cerebrovascular mortality (42-43), suggesting that sialic acids is a potential marker of vascular damage. Thus the release or shedding of sialic acids from tissues or cell surface to circulating blood might contribute to rising in serum sialic acids (44). Hence the estimation of other markers for a vascular damage is required in depressed patients and the follow up also is necessary to estimate the correlation between serum sialic acids, depression, and cardiovascular or cerebrovascular disorders. The increase in acute phase proteins in plasma of depressives and subsequent increase in serum sialic acids is supported by another evidence showed that the increase in total sialic acid in brain tumor patients decrease after treatment with anti-inflammatory drugs which in turn, decrease the acute phase proteins (45).

Plasma sialic acids is a marker of the acute phase reactants (35,66), and the acute phase reactants with sialic acids as a component of the oligosaccharide side chain being produced by the liver, stimulated by proinflammatory cytokines such as IL1, IL6 and tumor necrosis factor (47-48). These cytokine production are known to induce acute phase response (48). These interleukins were shown to be increased in depression (49). Hence the increase in these interleukins in depression may also be one of the possible causes for the increase in total sialic acid in the sera of depressed patients.

In humans, large quantity of sialic acids is found in acute phase proteins such as orosomucoid, α1-antitrypsin, haptoglobin and fibrinogen with negligible free sialic acids in the circulation (42, 50). Therefore, the changes in free sialic acid is not easy to predict. It has been demonstrated that insulin is a rapid, nonspecific, and dose dependent (physiological dose) inhibitor of the hepatic synthesis of acute phase proteins and that a decrease in insulin activity as a result of increased cortisol was reported in depression (51). These abnormalities may act as a possible mechanism for increasing sialic acid concentration.

References

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