Original Article

Increased Serum Sialic Acids in Depression.

Hussein Kadhem Abdul Hussein (PhD)*
Saadoon A. Isa (PhD)**
Ghassan A. Al-Shamma (PhD)**

Summary:

J Fac Med Baghdad
2007; Vol. 49, No.2
Received Jan. 2007
Accepted May 2007

***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 BSA in the depressed patients as compared with healthy controls.*
***Conclusions**: It could be concluded that TSA and BSA 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.*

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

(*) Department of Chemistry, College of Science , University of Karbala.
(**) Department of physiological Chemistry, College of Medicine AL-Nahrain University.

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 drown 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[28]as modified by (George *et al* 1981) [29] :

***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) [30]: 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 a standard curve developed from a standard sample of n-acetyl neuraminic acid and use of the following formula:

$$LSA\ mg/dL = \frac{x * 100000}{y * 50 * 1000} \text{ ----------(1)}$$

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):

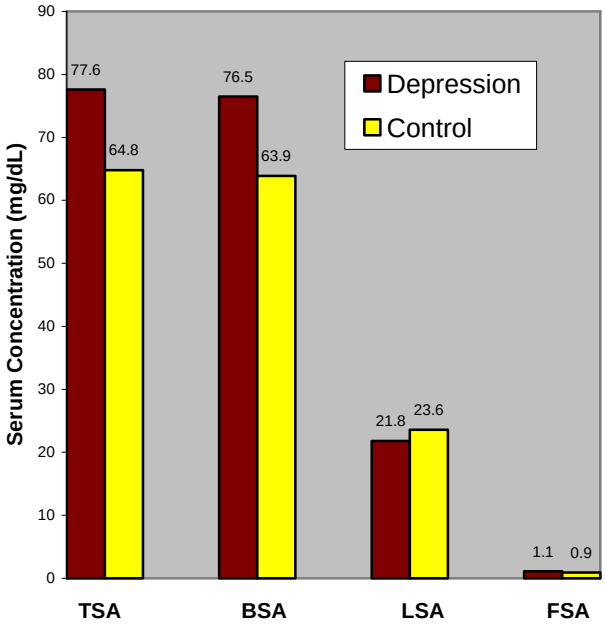
Chromogen formed by periodate oxidation is destroyed at 37˚C, whereas the chromogen obtained from the oxidation of the glycosidically bound sialic acid is stable at this temperature [29]. BSA was estimated using the protocol mentioned for TSA except that the oxidation step was carried out at 37˚C not 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.

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 acids 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 serum 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 [35,46], 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

1. Kenneth B. Wells (1985): Depression as a tracer condition for the National Study of Medical Care Outcome: Background Review. 1st Ed. Rand Corporation.

2. Sadock J.M. and Sadock V.(2000): Kaplan and Sadock's Comprehensive Textbook of Psychiatry. Vol. 1, 7th Ed. Lippincott Williams and Wilkins. USA.

3. Katon W., and Sullivan M.D. (1990): Depression and chronic medical illness. J.Clin.Psychiatry. 56 (Suppl.),3-11.

4. Romano J. and Turner J. (1985): Chronic pain and depression :does the evidence support a relationship ? Psychol. Bulletin . 97, 18-34.

5. Katon W., (1987): The epidemiology of depression in medical care. Int. J . Psych. 17, 93-111.

6. Howeland R. (1993): Thyroid dysfunction in refractory depression: implications for pathophysiology and treatment. J. Clin. Psychiatry. 54 : 47 - 54.

7. Jackson I. (1998): The thyroid and depression .Thyroid 8: 951-956.

8. Garvard J., Lustman P., and Clouse R. (1993): Prevalence of depression in adults with diabetes .An epidemiological evaluation . Diabetes Care. 16 : 1167-1178.

9. Kelly W. (1996): Psychiatric aspects of aspects of Cushing's syndrome. Q. J. M. 89 : 543-551.

10. Musselman D.(1998): The relationship of depression to cardiovascular disease epidemiology, biology, and treatment. Arch . Gen .Psychiatry 55: 580-592.

11. Kotila M., Numminen H. Waltimo O. (1998): Depression after Stroke: results of the FINNSTROKE Study. Stroke . 29 :368-372.

12. Shauer R. (1985): Sialic acids and their role as biological markers. Trends Biochem. Sci. 10 : 357.

13. Shauer R. (1982): Adv. Carbohydrate Chem. Biochem. 40: 181-204. Cited by Shauer R. (1985).

14. Yarema K., Goon S., and Bertozzi C. (2001): Metabolic selection of glycosylation defects in human cells. Nat. Biotechnol. 19 : 553-558.

15. Painbeni T. (1997): Plasma sialic acid as marker of the effect of the treatment on metastatic colorectal cancer." Eur .J .Cancer. 33 : 2216 - 2220.

16. El-Abbadi M (2001): Ganglioside composition and histology of a spontaneous metastatic brain tumor in the VM mouse. Br. J. Cancer 85 : 285-292.

17. Chen Z., Ito K., Fujii S. (1996): Roles of dopamine receptors in long-term Depression: enhancement via D1 receptors and inhibition via D2 receptors . Receptors-Channels .4 : 1-8.

18. Garcia-Sevilla J., Escriba P., Ozaita A. (1999): Up-regulation of immunolabeled alpha 2A-adrenoreceptors, Gi coupling proteins and regulatory receptor kinase in the prefrontal cortex of depressed suicides. J .Neurochem. 72 : 282-291.

19. Wichers M., and Maes M (2002): Cytokines-induced depression. The psychoneuroimmuno-pathophysiology of cytokine-induced depression in humans. Int .

Neuropsychopharmacol. 5 :375-388.

20. Widmer J., Henrotte J. ,Raffin Y., et al (1995): Relationship between erythrocyte magnesium, plasma electrolytes and cortisol, and intensity of symptoms of major depressed patients. J. Affect. Disord. 34: 201-209.

21. Widmer J., Mouthon D., Raffin Y. (1997): Weak association between blood sodium, potassium, and calcium and intensity of symptoms in major depressed patients. Neuropsychobiology 36: 164 -171.

22. Maes M., Meltzer H., Cosyns P. (1993): An evaluation of basal hypothalamic-pituitary axis function in depression: results of a large scaled and controlled study. Psychoneuroendocrinology 18 : 607 - 620.

23. Upadayaya A., Pannell I., Cowen P., and Deaken J.(1991): Blunted growth hormone and prolactin responses to L-tryptophane in depression: a state-dependent abnormality. J. Affect . Disord. 21: 213-218.

24. Freedman S., Byers T., Barrett H. et al (1995): Plasma lipid levels and psychologic characteristics in men . Am.J.Epidemiology 141: 507-517.

25. Maes M., D'Haese P., ScharpeS. et al (1994): Hypozicaemia in depression. J . Affec t. Disord. 31 : 135 -140.

26. Maes M., Vandoolaeghe E., Neels H. (1997): Lower serum zinc in major depression is a sensitive marker of treatment resistance and of the immune /inflammatory response in that illness. Biol. Psychiatry 42 : 349-358.

27. Fernandez-Gonzalez M., Garcia-Unzueta M., Herran A. (1998): Trace elements in serum of psychiatric out patients .Quimica Clinica 17 : 208-212.

28. Svennerholm L. (1957): Quantitative estimation of sialic acids II. A colorimetric resorcinol-hydrochloric acid method. "Biochemica Biophysica Acta. 24 : 604-611.

29. George W., Jourdian L., and Roseman S. (1981): J .Biol .Chem. 246: 430-436.

30. Katopodis N., and Stock C. (1980): Improved method to determine lipid bound sialic acid in plasma or serum. Res . Common . Chem . Path.Pharm. 30 : 171-180.

31. Song C., Lin A., Bonaccorso S., et al (1998): The inflammatory response system and the availability of plasma tryptophane in patients with primary sleep disorders and major depression. J .Affect.Disord. 49 : 211-219.

32. Reif W., Pilger F., Ihle D., et al (2001): Immunological differences between patients with major depression and somatization syndrome. Psychiatry Res. 105: 165-174.

33. Maes M., Verkerk R., Vandoolaeghe E. (1997): Serotonin-immune interactions in major depression: lower serum tryptophane as a marker of immune-inflammatory response. Eur . Arch . Psychiatry Clin. Neurosci. 247:154-161.

34. Huang T. (2002): Lower serum albumin levels in patients with mood disorders. Chang Gung Med. J. 25 : 509-513.

35. Taniuchi K., Chifu K., and Hayashi N.(1981): A new enzymatic method for the determination of sialic acid in serum and its application for a marker of acute phase reactants. Kobe J . Med . Sci . 27: 91-102.

36. Ng S., and Dian J. (1976): The natural occurrence of sialic acids. In Biological Role of Sialic Acid. Rosenburg A., Schengrund S., Eds. N.Y. Plenum pp. 59-102.

37. Costello M., Barry A., Fiedel B., and Gerwurz H. (1979): Inhibition of platelet aggregation by native and desialized alpha-1 acid glycoprotein. Nature 281 : 677-678.

38. Chandrasekhar N., Warren L., Osbahr A.J., and Laki K. (1962): Role of sialic acid in fibrinogen. Biochem. Biophys. Acta. 63 : 337-339.

39. Schnitzer J., Pinney E., (1992): Quantitation of specific biding of orosomucoid to cultured microvascular endothelium: role in capillary permeability. Am.J.Physiol. 32: 48-55.

40. Orekhov A., Tertov V., Mukhin D. (1991): Desialylated low density lipoprotein: naturally occurring modified lipoprotein with atherogenic potency. Atherosclerosis. 86 : 153-161.

41. Rogers M., Wiliams D., Niththananthan R. (1992): Decrease in erythrocyte glycophorin sialic acid content is associated with increased erythrocyte aggregation in human diabetes. Clin.Sci. 82 : 309 - 313.

42. Lindberg G., Eklund G., Gullberg B. (1991): Serum sialic acid concentration and cardiovascular mortality. Br. Med . J. 302 :143-146.

43. Lindberg G.,Rastam L., Gullberg B. (1992): Serum sialic acid concentration predicts both coronary heart disease and stroke mortality : multivariate analysis including 54,385 men and women during 20.5 years follow up. Int. J . Epidemiol. 21: 253-257.

44. Yokoyama H., Jenson J., Myrup B. et al (1996): Raised serum sialic acid concentration precedes onset of microalbuminuria in IDDM: A 10-year follow-up study. Diabetes Care. 19: 435-440.

45. Yahya Y.Z.Farid, Hussein K.A. Hussein, and Sarmad Aziz (2004): Evaluation of total serum sialic acid and lipid associated sialic acid in brain tumor patients. Iraqi J. Med. Sci. 3 : 29-31.

46. Pickup J., Mattock M., Chusney G. and, Burt D. (1997): NIDDM as a disease of the innate immune system: association of acute phase reactants and interlukin-6 with metabolic syndrome X. Diabetologia 40:1286-1292.

47. Kushner I. (1993): Regulation of the acute phase response by cytokines. Perspect. Biol. Med. 36 : 611-622.

48. Baumann H. and Gauldie J. (1994): The acute phase response. Immunol. Today. 15: 74-80.

49. Berk M., Wadee A., Kuschka R. et al (1997): Acute phase proteins in major depression. J. Psychosom. Res. 43 : 529-534.

50. Carter A., and Martin N. (1962): Serum sialic acid in health and disease. J. Clin. Pathol. 15: 69-72.

51 – Muck-Seler D , Pivac N , Sagud M (2002) . The effect of paroxetine and tianeptine on biochemical markers in major depression . Prog. Neuropsychopharmaco. Biol. Psychiatry 26 : 1235- 1243.