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Sialic acid, homocysteine and CRP: Potential markers for dementia

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ABSTRACT

To investigate whether sialic acid could discriminate between healthy age matched controls and patients with dementias of the Alzheimer's type (AD), and pure vascular dementia (VaD). 27 patients and 51 controls were administered the Mini-Mental State Examination (MMSE) and had blood analyzed for levels of total sialic acid, total homocysteine (tHcy), and C-reactive protein (CRP). Significant differences were found between the mean MMSE scores for patients with dementia compared with controls. Sialic acid levels were significantly higher in patients with AD compared with controls and homocysteine levels were higher in VaD. Sialic acid levels discriminated between patients with dementia of the Alzheimer's type and healthy controls only. The MMSE could discriminate between controls and patients with dementia but not between the subtypes and homocysteine was significant for patients with VaD.

Alzheimer's disease is considered the most prevalent type of dementia among the elderly [18]. Its etiology is unknown, there is no cure for this disease but early detection, and intervention may prolong and improve the quality of life of the individual [2]. In this regard there has been worldwide collective effort to establish a biomarker for the disorder. One avenue currently being pursued is that of inflammatory markers. This is because inflammation plays significant roles in early events leading to the pathogenesis of AD. Thus targeting these initial events could lead to a means of early intervention. One of the main players in early pathological events is cytokines [15]. Others include C-reactive protein about which much has been written [9] and sialic acid. Due to the relationship of cytokines and AD pathology volumes of information have been generated about the possible use of circulating cytokines in AD and other dementias as biological markers. However, for the most part, the use of circulating cytokines alone has not provided the expected outcomes and the data is quite scattered [4,19,21]. In view of the fact that risk factors associated with cardiovascular disorders such as hypertension, diabetes and metabolic syndrome are currently being linked with dementia it is of interest that elderly subjects displaying both metabolic syndrome and elevated cytokines displayed accelerating cognitive aging [30,29].

African Caribbean populations may have a raised risk of dementia because hypertension, diabetes and cerebrovascular disease are common among them [24]. High rates of diabetes have been reported in Barbados [10] and in Trinidad there is an increased risk of diabetes in East Indians [14] and this too might contribute to increased levels of dementia. Serum or plasma sialic acid (N-acetyl neuraminic acid), an inflammatory marker, has recently been shown to be a strong predictor of cardiovascular mortality [11,12]. It has been recently established that serum sialic acid is a potent cardiovascular and renal risk factor as it is increased in cerebrovascular disease and in patients with micro- and macrovascular complications of diabetes [17]. The links between sialic acid and disorders, which are currently considered as high risk factors for dementia, suggests that it may be a useful predictor of persons who are susceptible to developing dementia. To date in spite of the fact that it is now known that inflammation plays a role in Alzheimer's disease pathology there are no reports on sialic acid levels in such patients.

The Mini-Mental State Examination (MMSE) [6] which is currently used to establish cognitive levels in populations is not able to discriminate between vascular dementia and dementias of the Alzheimer's type. In order to offer care to persons who could respond to treatments aimed at slowing down progression it is necessary to establish means to discriminate among different types of dementias. This is indeed a high priority area in the field of dementia research.

High circulating concentrations of the amino acid homocysteine (tHcy) is an independent risk factor for dementia and is associated with vascular disease [23], CRP an acute phase protein is considered to have a link to cardiovascular disorders and is an inflammatory

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marker which has been investigated in relationship to the development of certain dementias [9]. Patients may be initially diagnosed with dementia of the Alzheimer's type and then develop a vascular component or are initially diagnosed with vascular dementia and develop an AD component.

To this end the present study investigates the MMSE scores, levels of tHcy, CRP and sialic acid in a well-defined cohort of dementia patients and healthy age matched controlled seniors. The overall goal of this study was to determine whether sialic acid is elevated in patients with dementia and whether it could further be used to differentially diagnose dementias. Sialic acid levels are also compared along with homocysteine and C-reactive protein levels.

There were 27 patients enrolled in this study 12 males and 15 females that either presented with a complaint of memory impairment or exhibited memory impairment during routine psychiatric evaluation at a specialist psychiatry memory clinic in a hospital setting and a private psychiatry clinic. The MMSE was administered and all patients were subjected to interview, physical examination and neurological examination. These cases met the criteria of the Diagnostic and Statistical Manual of Mental disorders, Fourth Edition TR (DSM IV TR) for dementia. Laboratory tests and brain computed tomography CT or MRI were done. Patients were further classified as follows: (1) possible or probable Alzheimer's disease as defined by National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS–ADRDA) guidelines for AD; (2) probable vascular dementia according to National Institute of Neurological Disorders and Stroke and Association Internationale pour la Recherche et l'Enseignement en Neurosciences (NINDS–AIREN) criteria for VaD. This investigation included 51 healthy age matched controls (defined as individuals aged 65 and over according to the US Census Bureau). These individuals were enrolled on a consecutive basis.

Clinical variables were evaluated and blood testing was done for plasma tHcy which was determined on the Abbot AxSym using a fluorescence polarization immunoassay (FPIA), serum CRP concentrations were measured using the Tina-Quant sCRP (Latex) high sensitive immunoturbidimetric assay on the Roche/Hitachi 912 Automatic Analyzer (Roche Diagnostics, GmbH, D-68298 Mannheim) and serum sialic acid was measured by spectrophotometric assay using standard chemicals and reagents. In this method, a protein precipitate containing sialic acid will react with diphenylamine producing a purple color, which is quantitatively measured on a spectrophotometer at 540 nm [28]. The healthy volunteers all had MMSEs done and blood was also taken and analyzed for levels of serum CRP, plasma tHcy and serum sialic acid. All volunteers were required to fill out a questionnaire in order to obtain demographic information. The clinical/biochemical characteristics of both groups were compared.

The dementia patients had a mean age of 78.0 years, and the control group had a mean age of 77.0 years. The mean MMSE score of the patients with dementia was 15.23 ± 1.95, range 1–24, for the control group the mean MMSE score was 28.71. For the dementia patients the main clinical diagnoses were AD, 18 (67%) and VaD, 9 (33%); When the controls were compared with all patients as a group, the MMSE and sialic acid was significantly different, with MMSE scores being higher in controls and sialic acid levels were lower. These patients were further processed by dividing them up into specific diagnoses and comparing results. A comparison of the subgroups for the different biomarkers is shown in Table 1. Patients with AD had significant differences in the MMSE scores and sialic acid scores, but not tHcy and CRP values when compared with controls. In patients with VaD however, significant differences were obtained for the MMSE scores and tHcy but not for sialic acid or CRP. Table 2 shows the p values from independent t-tests of cases compared with controls for MMSE scores, sialic acid, tHcy and CRP levels. The presence of amyloidosis or cerebral amyloid angiopathy (CAA) in the patients with VaD was not known. The true incidence and prevalence are hard to specify, as definite CAA is a pathologic diagnosis typically obtained post-mortem [5,31].

Table 1
Comparison of subgroups.

Subjects	Indicators (mean ± SEM)			
	MMSE (/30)	Hcy (μmol/L)	CRP (mg/L)	Sialic acid (mg/100 mL)
Controls	27.52 ± 0.45	9.85 ± 0.32	3.16 ± 0.40	65.65 ± 2.10
AD	16.67 ± 1.47	10.35 ± 0.52	0.80 ± 0.17	77.17 ± 3.90
VaD	16.11 ± 1.78	11.66 ± 1.07	2.86 ± 1.05	65.60 ± 4.58

AD: Alzheimer's disease; VaD: vascular dementia; MMSE: Mini-Mental State Examination; tHcy: total homocysteine; CRP: C-reactive protein.

Table 2
Comparison of biomarkers showing p values.

Comparisons	Parameters			
	MMSE	Sialic	Hcy	CRP
Controls vs. AD	0.000	0.041	0.410	0.083
Controls vs. VaD	0.000	0.991	0.036	0.501
AD vs. VaD	0.821	0.148	0.224	0.417

Significance $p < 0.05$; AD: Alzheimer's disease; VaD: vascular dementia; MMSE: Mini-Mental State Examination; tHcy: total homocysteine; CRP: C-reactive protein. The underline values indicate p-values that were significant.

In recent times it has been recognized that risk factors for cardiovascular disease are also risk factors for dementia of the Alzheimer's type, disease conditions such as hypertension and diabetes as well as abnormal levels of homocysteine and CRP have been associated with Alzheimer's disease [33]. The literature reports for CRP however have been conflicting [4,9]. In the present study CRP was unable to distinguish between subtypes of dementia. Recent work [8] suggests that in the oldest old (patients older than 90 years) high CRP levels were associated with increased odds of all-cause dementia particularly in females. It is noted however that our patients were younger. The average age of our patients was 78 years.

Sialic acid is a component of cell membranes [32] and elevated levels may indicate excessive cell membrane damage, but more specifically to the cells of vascular tissue. If there is damage to vascular tissue, this leads to ischemia and this ischemia is most visible in the smallest blood vessels, including those of the retina of the eyes, kidneys, heart and brain. It is this ischemia that leads to conditions including, but not limited to retinopathy, nephropathy and neuropathy. In addition, sialic acid can be used as a measure of the acute phase response because many of the proteins of the immune response are actually glycoproteins and these glycoproteins have sialic acid as the terminal sugar on their oligosaccharide chain [16].

Serum sialic acid is a protein bound carbohydrate and occurs in combination with monosaccharides like galactose and mannose. Ninety percent is bound and almost none free [7]. In serum they are generally bound to acute phase proteins [13]. There are several possible explanations for the increase in serum sialic acid concentrations. Several research studies have shown that the concentration of sialic acid in serum is elevated in pathological states when there is damage to tissue, tissue proliferation and inflammation, the latter of which has in recent times reemerged as an important aspect of the pathogenesis of Alzheimer diseased [7]. Our findings suggest that elevations in serum sialic acid levels could be related to AD pathology. In this regard it is of interest that a recent study [26] has demonstrated that reduction in sialic acid protects PC 12 cells from B amyloid toxicity. From a speculative point of view the elevated levels in sialic acid may reflect an increase in the deposition of B amyloid. Further studies are necessary to elucidate the relationship between elevated sialic acid levels and ongoing AD pathology.

A review of the literature with respect to sialic acid has hinted at an ethnic influence with respect to levels of this analyte in some conditions for example the association between total sialic acid and nephropathy was not observed in Indian and Malay ethnic groups, some authors highlight the unpredictable nature of sialic acid as a potential marker of the acute phase response in different ethnic groups.

In Trinidad and Tobago, the ethnic composition is East Indian 40.3%; African 39.6%; mixed 18.4%; and 1.7% belong to other ethnic groups [1] Studies have found ethnic differences in the incidence of AD. The influence of ethnic differences in our results may however be limited since in our study only one patient was of East Indian descent in spite of the fact that our population comprises about 39.6% persons of African descent and 40.3% persons of East Indian descent. This finding need to be explored in future studies where factors such as diet in different ethnic groups might be important. Curcumin for example an ingredient in Indian curry has been reported to be protective of Alzheimer disease [20].

In our study AD was the most common type of dementia followed by VaD a common finding worldwide. The finding that sialic acid levels were significantly higher in patients with AD compared to controls and not different with respect to VaD is unlike the results for tHcy where notable differences were found between VaD and controls. This suggests that there may be different mechanisms at work in the pathogenesis of the two conditions. The role of homocysteine in the development of dementia has engaged active debate in the literature [25,23]. Our results points toward a vascular mechanism such as altering LDL receptor function increasing atherosclerotic plaque formation and prothrombotic and procoagulant effects [22,27]. The majority of markers for dementia being commercialized are located in the CSF. The use of CSF to differentially diagnose dementias has numerous disadvantages and would be considered quite impracticable for routine screening. Our findings for microglial antibodies [3] and sialic acid another serum analyte add to the growing number of biomolecules to be considered as candidate biomarkers for Alzheimer disease.

One of the limitations of sialic acid is that it may also be elevated in several conditions such as malignancies of the larynx and eye as well as colorectal carcinoma. It is also elevated in inflammatory diseases such as liver cirrhosis and Bechet's disease. The fact that sialic acid has been suggested as a marker of chronic inflammation, lends supports for a role for inflammation in dementia but needs further study.

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