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Acetylcholinesterase activity measurement and clinical features of delirium

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Running head: AChE activity and delirium

ABSTRACT:

Aims: Cholinergic deficiency is commonly implicated in the pathophysiology of delirium. We aimed to investigate the relationship between directly measured serum AChE activity and (1) clinical features of delirium and (2) outcomes, among older hospital patients with delirium.

Methods: Hospitalized patients with delirium were recruited and delirium motor subtype, severity and duration of delirium were measured. Serum AChE activity was measured using a colorimetric assay.

Results: The mean AChE activity for the whole sample was 2.46 $\mu\text{mol}/\mu\text{ml}/\text{min}$ (SD 1.75). Higher AChE activity was associated with increased likelihood of hypoactive delirium rather than the hyperactive or mixed subtype (OR 1.98, CI 1.10-3.59).

Conclusion: Higher AChE activity was associated with hypoactive delirium, but did not predict outcomes. Simple enhancement of cholinergic neurotransmission may not be sufficient to treat delirium

Key words: Acetylcholinesterase activity, acetylcholine, delirium, cholinesterase inhibitors, aged, delirium motor subtype

INTRODUCTION

Delirium is an acute neuropsychiatric syndrome of acutely altered mental status characterised by inattention, other cognitive deficits, altered level of arousal, psychotic features, and other disturbances. Older people are at increased risk of delirium and the prevalence of delirium in older people admitted to medical wards has been reported as 18-35% [1]. Delirium is associated with worse outcomes, including mortality, incident dementia, length of stay and institutionalization [2] but is often under recognised, particularly its hypoactive subtype[1]. The pathophysiology of delirium is currently not well understood [3]. Abnormalities in acetylcholine neurotransmission are hypothesised to play a role in initiation and maintenance of delirium [4]

Evidence for the cholinergic hypothesis in delirium comes from a number of areas. First, cholinergic pathways in the basal and rostral forebrain are involved in conscious awareness, attention and working memory, areas that are often affected in delirium [5]. Second, dysfunction of cholinergic neurons and neuronal loss has been found to lead to low levels of the neurotransmitter acetylcholine (ACh) in both dementia and delirium[6]. Thirdly, delirium has been demonstrated in animals through both the administration of anticholinergic drugs [7] and following septic challenge in animals with cholinergic neurons selectively removed [8]. Fourth, drugs with anticholinergic effects are associated with delirium in humans [9] as well as being associated with a higher risk of developing both short and long term cognitive impairment [10]. Finally, serum anticholinergic activity has been associated with delirium [11] though not all studies confirm this[12].

Acetylcholinesterase (AChE) inactivates acetylcholine by hydrolysing it to form acetate and choline. Reduced serum AChE activity from peripheral blood has been associated previously with development of delirium in a medical [13] and surgical population [14 15]. Reduced serum AChE activity in patients without delirium is associated with higher inpatient mortality [13] and frailty [16]. A role for AChE inhibitors in the treatment and prevention of delirium has been proposed and this has been supported by a

number of case reports and small studies [17]. More recently, randomised controlled trials have had negative results [18 19] and a major study had to be prematurely halted due to raised mortality in intensive care patients treated with rivastigmine [20], although this was conducted in a younger population in the intensive care unit.

Given this uncertainty a greater understanding of AChE enzyme activity may help further elucidate our understanding of the pathophysiological mechanisms underlying delirium. Here we aimed to describe differences in directly measured serum AChE activity and their relationship with (1) clinical features of delirium and (2) outcomes, among older hospital patients with delirium. We hypothesise that older patients with delirium would have a deficit in cholinergic neurotransmission, so we would expect the acetylcholinesterase enzyme activity to be downregulated to compensate for this. We hypothesize that hypoactive delirium would be associated with a greater degree of cholinergic deficit, and hence a greater secondary downregulation of the enzyme, or alternatively an increased enzyme activity driving the cholinergic deficit.

METHODS

Study Sample

The sample population for this study was newly-admitted medical patients to the Queen Elizabeth Hospital Birmingham, aged over 70 years, presenting with delirium during the period March 2013 to February 2014. Eligible patients were screened for and diagnosed with delirium by a geriatrician (TAJ) using the Diagnostic and Statistical Manual of Mental Disorders (4th ed., text rev.; DSM-IV-TR; American Psychiatric Association, 2000) criteria. The screening used the Confusion Assessment Method (CAM) [21], Abbreviated Mental Test Score (AMTS) [22], the Digit Span test, and a detailed review of the medical notes. Exclusion criteria were: inability to communicate through severe sensory impairment, non-competence in the English language and imminent death. The National Research Ethics Service Committee of Yorkshire and Humberside approved the study (Ref: 12/YH/0534) and consent, or consultee declaration, was obtained from all participants. The study sample was 87 patients with confirmed delirium.

Study variables

Demographics were collected for all patients. Severity of illness was measured using APACHEii [23] and frailty was measured using the Rockwood Clinical Frailty Scale [24]. Dementia was defined using the Informant Questionnaire of Cognitive Decline in the Elderly (IQCODE) where a score of >3.82 represented dementia [25] and information was gathered from consultees. Delirium severity was measured using the Delirium Rating Scale Revised version (DRSR-98) [26]. The delirium rating scale is rated from 0 to 39 across 13 domains, with a higher value representing more severe delirium and incorporates reports from caregivers. Patients were reviewed every 48 hours to ascertain delirium length. These assessments were continued throughout hospital admission, with medical note review and nurse assessment to assess for delirium in the intervening time. Delirium subtype was classified by clinical assessment using elements of the DSR-R-98 and recognized classifications [27 28] into hypoactive, hyperactive, or mixed subtypes. Patients were reviewed directly at 3 months. Poor outcome was classified

as death, new institutionalisation or persistent delirium at 3 month follow up. To measure anticholinergic drug burden the Anticholinergic Drug Scale (ADS) was calculated for each participant based on the recorded pre-admission drugs [29]. All assessments were carried out by a single trained expert assessor (TAJ)

Acetylcholinesterase (AChE) Measurement

Venous blood samples were taken the morning of recruitment and immediately separated by centrifugation to serum. Serum samples were then frozen and stored at -80°C until analysis. Serum AChE activity was measured using the Ellman method [30] with a commercially available kit (Abcam, Cambridge, UK). The substrate, acetylthiocholine iodide, was hydrolysed by AChE in the sample serum to produce thiocholine and acetate. Thiocholine was then reacted with 5,5'-dithio-bis-nitrobenzoic acid (DTNB) producing the yellow anion 5-thio-2-nitrobenzoic acid. 5-thio-2-nitrobenzoic acid was measured through colorimetric analysis. After an initial delay of 2 minutes the change in absorbance was monitored spectrophotometrically at 410nm every 60 seconds for 240 seconds. The change in absorbance per minute was then used to calculate AChE activity expressed as $\mu\text{mol/ml/min}$.

Statistical Analysis

Data were analysed using IBM SPSS Statistics for Windows (Version 20.0. Armonk, NY: IBM Corp.) , and normality of data was assessed using the Shapiro-Wilk test. Demographic and delirium characteristics were reported for the total cohort and by two binary variables; dementia status defined as IQCODE >3.82 and motor subtype (hypoactive vs mixed and hyperactive). By defining the motor subtypes in a binary category this isolates purely low arousal motor subtypes and compares this to motor subtypes with increased arousal. Statistical differences between groups were measured by chi squared for categorical variables, and Mann Whitney U test or Student's T test depending on normality. Correlation between AChE activity levels and delirium length was assessed using the Spearman Rank correlation.

Correlation between AChE activity levels and delirium severity was assessed using the Pearson correlation test.

Associations between AChE activity and the binary variables of outcome, mortality and delirium subtype (hypoactive compared to hyperactive and mixed) were measured by multivariate binary logistic regression. For subtype outcomes drug anticholinergic activity using the ADS score and dementia using the IQCODE score were controlled for. These were chosen as potential confounders due to their potential effect on cholinergic activity. For mortality and outcome variables; ADS score, age, frailty and disease severity, were controlled for due to their effect on outcome. Linearity was tested for using the Box-Tidwell test. Statistical significance was set at $p < 0.05$.

RESULTS

Screening identified 164 people with delirium, of whom 87 were recruited. 60 participants with delirium had a serum sample available. There was no difference in demographics between those recruited and those not (Figure 1). 37 (62%) had dementia, of whom five (8%) were on cholinesterase inhibitor drugs so were excluded from the final analysis, leaving a final study sample of 55. The mean age of the sample was 85.5 years (SD 6.15) and 61.8% of the sample was female. The median length of delirium was 4 days (IQR 5) and the mean delirium severity score (DRS-R98) was 17.3 (SD \pm 6.4). The hypoactive subtype was most common (34 patients, 62%) while 12 (22%) had the hyperactive subtype and 9 (16%) the mixed subtype. The mean AChE activity for the whole sample was 2.46 (1.7). Table 1 summarises these. Median activity for those on cholinesterase inhibitors was lower at 1.12 $\mu\text{mol}/\mu\text{ml}/\text{min}$, IQR 0.80, $p=0.03$).

Relationship between AChE activity and clinical features of delirium

AChE activity was not correlated with age ($r=0.04$, $p=0.77$). There were no differences between those with and without dementia in age, delirium severity, subtype, duration, and AChE activity.

There was no correlation between delirium severity ($r=0.07$, $p=0.59$) and AChE activity, and no correlation between AChE activity and length of delirium ($\rho=0.125$, $p=0.368$). A one-way between subjects ANOVA was conducted to compare the effect of AChE activity on the three motor subtypes.

There was a significant effect of AChE activity on the three motor subtypes ($F[2, 52] = 3.27$, $p = 0.046$). This is illustrated in Figure 2. Mean AChE activity was higher in the hypoactive subtype compared to those with mixed or hyperactive subtype (2.74 $\mu\text{mol}/\mu\text{ml}/\text{min}$ vs 1.75 $\mu\text{mol}/\mu\text{ml}/\text{min}$, $p=0.016$). Higher AChE activity was associated with the hypoactive subtype on univariate analysis (OR 1.77, CI 1.05-2.97, $p=0.03$). When controlling for anticholinergic drug activity by ADS score, increased AChE activity remained associated with the hypoactive subtype (OR=1.76, CI 1.05-2.96, $p=0.03$). The same was true when also controlling for both dementia, using IQCODE score, and ADS score (OR 1.98, CI 1.10-3.59,

p=0.025).

Relationship between AChE activity and delirium outcomes

Of the 55 in the study group 49 had full outcome data. At 3 months follow-up 17 of 49 cases had died with 7 of those dying in hospital. 29 of 49 cases were classified as having a poor outcome (mortality, new institutionalisation or persistent delirium). Univariate logistic regression showed no relationship between AChE activity and in-hospital mortality (OR 0.97, CI 0.61-1.65, p=0.98), 3 month mortality (OR=1.12, CI 0.81-1.55, p=0.56), or poor outcomes at 3 months (OR=1.16, CI 0.82-1.65, p=0.39). There remained no association with outcomes when controlling for ADS score, age, frailty and acute illness. See table 2.

DISCUSSION

We have demonstrated for the first time an association between elevated serum AChE enzyme activity and the hypoactive subtype of delirium. We have also demonstrated no difference between serum AChE enzyme activity and delirium severity, length of delirium or outcomes.

Strengths of the study are the prospective nature of recruitment with robust delirium diagnosis against standardised criteria, as well as subtype ascertainment by expert opinion. This has resulted in a representative cohort of older patients in hospital with delirium. Some limitations should be described. The blood was sampled at a specified time of day the day after recruitment, so it only represents a cross-sectional measure of enzyme activity. Delirium follow-up assessments were completed every 48 hours, and although review of medical notes as well as a care giver informant provided information on the interim period, detail on the duration and subtyping of delirium may have missed. The study was part of a larger cohort study of admissions with delirium, and although the study aims were to investigate differences in pathophysiology within delirium, the lack of a control groups without delirium is a further limitation. Serum AChE activity is a measure of peripheral enzyme activity and may not necessarily reflect central AChE activity. Analysis of AChE activity in cerebrospinal fluid was not possible on this cohort [31].

The measured median AChE activity in this study is consistent with previous results of AChE activity found in both medical [13] and surgical [14 15] patients with delirium. The finding of no association with outcomes is contrary to a previous reported study where lower AChE activity was associated with inpatient mortality in a sample of patients without delirium [13]. Increased AChE activity was associated with having the hypoactive subtype of delirium compared to the other subtypes and this is the first study to our knowledge to report this. AChE activity in delirium could be affected by anti-cholinergic drugs[32], or chronic low cholinergic conditions such as dementia [33] and when controlling for these factors the

association between increased AChE activity and hypoactive delirium subtype remained. This finding suggests that the symptoms seen in hypoactive delirium may be driven by higher serum AChE activity, and thus lower active acetylcholine, although causality is not clear. Cholinergic neuronal pathways in the basal and rostral forebrain are involved in conscious awareness, attention and working memory, cognitive domains that are predominantly affected in hypoactive delirium [5]. The lower AChE activity seen in delirium when compared to control may be due to a downregulation of the enzyme as a consequence of these reduced cholinergic neuronal pathways. By downregulating the enzyme there is increased ACh across the synapse. The higher AChE activity seen in only hypoactive delirium may well represent a failure to downregulate AChE, leading to a greater hypo-cholinergic state and hence the clinical signs seen in hypoactive delirium. It also suggests that the pathophysiology underlying the different subtypes of delirium may be different. Differences in AChE enzyme activity can be explained by genetic causes that could underlie differences in AChE activity according to distinct alleles [34] or single nucleotide polymorphisms [35]. This has been documented for butylcholinesterase [36], another non-specific cholinesterase found in humans. This may then suggest that genetics underlying AChE activity could play a role in determining whether patients develop the hypoactive or hyperactive subtype of delirium.

Our findings also suggest a possible explanation of why trials of AChE inhibitors used to treat delirium were not positive. None of the major studies conducted a subgroups analysis looking at the effects of treatment depending on subtype. This opens the question of whether those with the hypoactive subtype may have preferentially benefitted from AChE inhibitor treatment, given their higher baseline activity of AChE [18-20].

Future research should concentrate on further unpicking the relationship between the cholinergic system and the delirium pathotype. A simplistic understanding of the system, in which predictions that correction of cholinergic hypoactivity should prevent or reverse delirium is clearly not supported and a more

complex relationship between the cholinergic system and AChE activity with clinical features and outcomes of delirium is likely.

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Author Contributions: All authors made substantial contributions to the study concept and design. TAJ and HCM wrote the protocol and carried out the experiments. TAJ acquired subjects. TAJ and HCM contributed equally to the first draft and all authors contributed to analysis and interpretation of data and revision of the manuscript and provided final edits. TAJ is guarantor.

Conflicts of interest

The authors declare they have no conflicts of interest.

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Figure legends:

Figure 1: Flowchart of selection of study participants

Figure 2: AChE activity illustrated by motor subtype (mean, error bars SE mean). * $p < 0.05$ significant difference compared to hypoactive delirium. ANOVA between subtypes $f(2, 52) = 3.27, p = 0.046$

TABLES

Table 1: Demographic and delirium characteristics presented for the total cohort and by binary variables of dementia status (IQCODE > 3.82) and motor subtype (hypoactive vs mixed and hyperactive). Statistical differences between groups were measured by chi squared for categorical variables, and Mann Whitney U test or Student's T test depending on normality. DRSR-98=Delirium rating scale-revised-98; SD = standard deviation; IQR=interquartile range

	Total	Dementia		p	Motor Subtype		
	(n=55)	No dementia (n=23)	Dementia (n=32)		Hyperactive and mixed (n=21)	Hypoactive (n=34)	p
Age (years) mean \pm SD	85.5 (6.2)	84.9 (6.3)	86.0 (6.1)	0.66	85.5 (5.6)	85.7 (6.6)	0.72
Female n (%)	34 (62%)	16 (79%)	18 (56%)	0.31	11 (52%)	23 (68%)	0.25
Dementia (%, IQCODE>3.82)	32 (58%)	-	-	-	11 (52%)	21 (62%)	0.49
Length of delirium (days) median (IQR)	4.00 (5)	4 (11)	3 (3)	0.09	4 (3)	3.5 (7)	0.42
DRSR-98 mean \pm SD	17.3 (6.4)	15.5 (11)	19.0 (8)	0.49	19 (9)	17.5 (9)	0.54
Hypoactive subtype n (%)	34 (62%)	13 (57%)	21 (66%)	0.49	-	-	-
Total number of drugs, median (IQR)	8 (4)	8 (5)	7 (5)	0.88	8 (4)	7.5 (6)	0.54
Anticholinergic drugs scale score, median (IQR)	1 (3)	2 (3)	1 (2)	0.48	1 (3)	1 (2)	0.34
AChE activity (μ mol/ μ ml/min), mean \pm SD	2.46 (1.7)	2.03 (1.3)	2.77 (2.0)	0.33	1.75 (1.0)	2.74 (1.7)	0.016*

Table 2: Association between acetylcholinesterase (AChE) activity and clinical features of delirium and delirium outcome

Variable	Relationship
Age	$r=0.04$ ($p=0.77$)
Delirium severity	$r=0.07$ ($p=0.59$)
Length of delirium	$\rho=0.125$ ($p=0.368$)
Hypoactive subtype*	OR 1.98 (CI 1.10-3.59, $p=0.03$)
3 month mortality [%]	OR=1.14 (CI 0.80-1.61, $p=0.48$)
3 month poor outcome [%]	OR=1.12 (CI 0.80-1.58, $p=0.50$)

r =Pearson's correlation coefficient; ρ = Spearman's rank correlation coefficient; OR=odds ratio;

CI=95% confidence interval. * Controlling for ADS score and dementia, % controlling for ADS score,

age, frailty and disease severity. Poor outcome defined as mortality or new institutionalisation. For all

regression analysis linearity was proven using the Box-Tidwell test.

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