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The effect of electroconvulsive therapy (ECT) on serum tryptophan metabolites

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ABSTRACT

Background: Prior studies suggest that activation of the tryptophan catabolism via the kynurenine pathway by proinflammatory cytokines may be involved in the pathophysiology of depression. Electroconvulsive therapy (ECT) is an effective treatment for major depression (MD) with immunomodulation as one of the proposed modes of action.
Objective: The aim of this study was to investigate serum concentrations of tryptophan and kynurenine pathway metabolites in MD patients and healthy controls, and to explore the effect of ECT on components of the kynurenine pathway.
Methods: The study included 27 moderately to severely depressed patients referred to ECT. Blood samples were collected prior to treatment and after the completed ECT-series. Baseline samples were also collected from 14 healthy, age- and sex-matched controls. Serum concentrations of tryptophan, kynurenine, 3-hydroxykynurenine (HK), kynurenic acid (KA), xanthurenic acid (XA), anthranilic acid (AA), 3-hydroxyanthranilic acid (HAA), quinolinic acid (QA), picolinic acid (Pic), pyridoxal 5′-phosphat (PLP), riboflavin, neopterin and cotinine were measured.
Results: Patients with MD had lower levels of neuroprotective kynurenine-pathway metabolites (KA, XA and Pic) and lower metabolite ratios (KA/Kyn and KA/QA) reflecting reduced neuroprotection compared to controls. The concentration of the inflammatory marker neopterin was increased after ECT, along with Pic and the redox active and immunosuppressive metabolite HAA.
Conclusion: In this pilot study, we found increased concentrations of inflammatory marker neopterin and putative neuroprotective kynurenine metabolites HAA and Pic in MD patients after ECT. Further research in larger cohorts is required to conclude whether ECT exerts its therapeutic effects via changes in the kynurenine pathway.

Introduction

Major depression (MD) is a severe and potentially life-threatening psychiatric illness that accounts for a large part of the overall global burden of disease [1]. The neurobiology of depression is complex and not fully understood [2]. However, it has been shown that MD often is associated with increased levels of pro-inflammatory cytokines, suggestive of a mild to moderate immune and inflammation activation [3,4].

The kynurenine pathway of tryptophan metabolism [5] (Fig. 1) has been proposed as a link between inflammatory processes and depressive symptoms [6,7]. The essential amino acid tryptophan is mainly (90%) metabolised to kynurenine (Kyn) and a small

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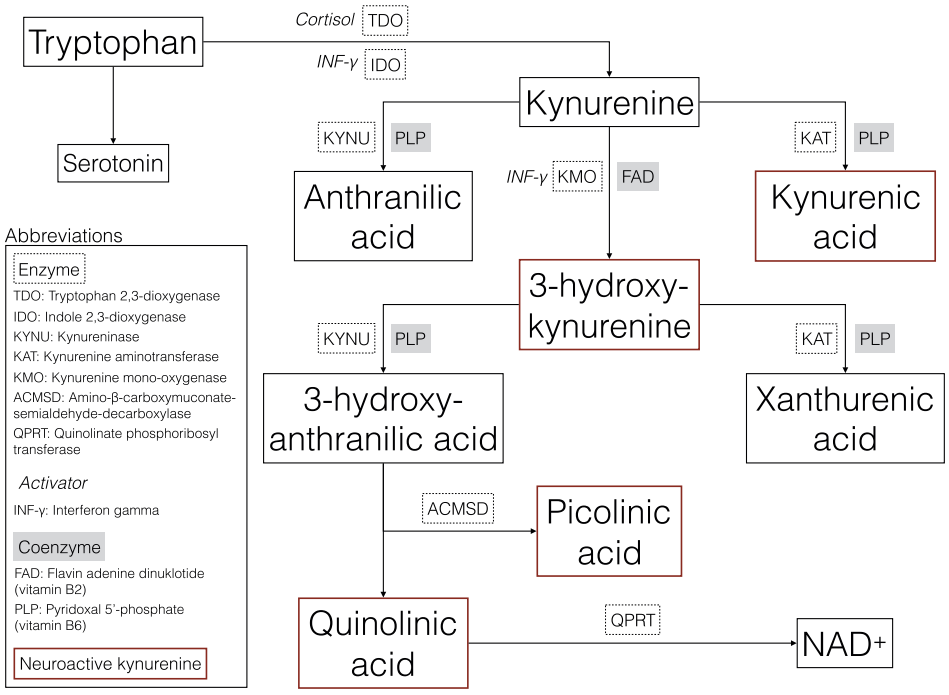

Fig. 1. The kynurenine pathway of tryptophan metabolism.

portion serves as precursor of serotonin. Conversion of tryptophan to kynurenine is regulated by tryptophan 2,3-dioxygenase (TDO) and indole 2,3-dioxygenase (IDO). The activity of IDO is stimulated by proinflammatory cytokines, especially interferon gamma (INF-γ), but also tumor necrosis factor alpha (TNF-α) and interleukin-6, whereas TDO is activated mainly by cortisol [8]. Through activation of IDO, inflammation leads to enhanced catabolism of tryptophan via the kynurenine pathway. The kynurenine to tryptophan ratio (KTR) functions as a proxy measure of INF-γ mediated activation of cellular immunity and this ratio has been shown to correlate positively with the concentration of other immune markers, like neopterin an established marker of cellular immune activation [9]. However, while conversion of tryptophan towards kynurenine is induced by both IFN-γ, through up-regulation of IDO, and by TDO, formation of neopterin is induced by IFN-γ only. Thus, circulating concentrations of neopterin are considered more specific to immune activation than is KTR. Kyn is metabolised further by the enzyme kynurenine aminotransferase (KAT) to kynurenic acid (KA), an *N*-methyl-D-aspartate receptor (NMDAr) antagonist and neuroprotective agent [8], or by kynurenine 3-monooxygenase (KMO) to 3-hydroxykynurenine (HK). HK is then metabolised through 3-hydroxyanthranilic acid (HAA) to either picolinic acid (Pic) or quinolinic acid (QA). Both HK and the NMDAr agonist QA are thought to exert neurotoxic effects [8]. Like IDO, KMO is activated by proinflammatory cytokines, directing metabolism through the neurotoxic branch of the kynurenine pathway and thus disrupting the balance between neuroprotective KA and the neurotoxic metabolites HK and QA [8,10]. Several steps in the kynurenine pathway are dependent on the coenzymes pyridoxal 5′-phosphate (PLP), the active form of vitamin B6, and flavine adenine dinucleotide (FAD), the active form of riboflavin (vitamin B2) [11] (Fig. 1). The serum level of these vitamins is affected by smoking [12]. Cotinine, a metabolite of nicotine, is a commonly used serum marker of recent nicotine exposure [13].

The status of the kynurenine pathway can be described by a set of ratios starting with KTR as a marker of the first and rate-limiting step catalysed by INF-γ-responsive enzyme, IDO. The direction of the Kyn breakdown and the flux through the downstream enzymes, KAT and KMO, are reflected by KA/Kyn and HK/Kyn, while KA/HK and KA/QA reflect the balance between the two main branches of the pathway [14]. Several studies have shown that MD patients have significantly lower plasma concentration of KA and lower KA/KYN and KA/QA than healthy controls, indicating altered balance in favour of neurotoxic metabolites [6,14–17]. The ratio XA/HK is a useful marker for vitamin B6 [18], an important coenzyme in several steps in the kynurenine pathway. Finally, the enzyme aminocarboxymuconate semialdehyde decarboxylase (ACMSD) limits QA formation by competitive production of the putative neuroprotective metabolite Pic. It has been suggested that QA might induce suicidal symptoms by affecting glutamate neurotransmission [19]. Furthermore, a study assessing the CFS and plasma Pic to QA ratio in suicide attempters supported the hypothesis that a reduced ACMSD activity underlies excess of neurotoxic QA production observed in patients exhibiting suicidal behavior [20]. The ratio of Pic and QA (Pic/QA) can be used as an estimate of ACMSD activity.

Electroconvulsive therapy (ECT) is considered the most effective treatment option for severe or treatment resistant MD [21]. It has been suggested that ECT may act by modulating immunological mechanisms [22–24]. Studies on how ECT impacts the immune system have indicated that a single session of ECT might induce an acute activation of immune response [25–27], while repetitive ECT treatment can down-regulate proinflammatory markers [27–29]. Through this immunomodulating effect, ECT might also affect the tryptophan metabolism [24]. Studies suggest that ECT in MD patients might shift the tryptophan metabolism towards metabolites with neuroprotective properties, with increase in KA and KA/HK [22] and decrease in QA after treatment with ECT [16]. However, other studies found no significant changes in KA [30] or in KYN, KA and KA/KYN [17].

The aim of this study was to investigate serum concentrations of tryptophan and a large panel of kynurenine pathway metabolites in MD patients referred to ECT in comparison with healthy controls and to explore the effect of ECT on the kynurenine pathway over a whole course of ECT.

Material and methods

Study design

In this prospective, observational study we collected blood samples and assessed the severity of depressive symptoms in major depression patients before and after a series of ECT. Additionally, the study included a group of age- and sex-matched healthy controls that contributed with the same baseline data. The study protocol has previously been reported in detail [31].

Ethical considerations

The study was approved by the Regional Committee for Medical Research Ethics in South East Norway (2013/1032). All participants provided informed written consent to participate in the study.

Participants

Between September 2013 and November 2016, 30 patients and 14 age- and sex-matched healthy controls from Hordaland, Norway, were included into the study. Patients (age > 18) were referred to and accepted for ECT because of a moderate to severe uni- or bipolar depressive episode with or without psychotic symptoms. The diagnosis was established by the treating clinician based on a clinical interview and information from medical records on symptoms, course of illness, family history, and past treatment. The following criteria were used for exclusion of patients: ECT within the last 12 months and moderate kidney failure (serum creatinine > 120 μmol/L). Data on clinical characteristics were recorded along with medication use both before and after treatment. Healthy controls were recruited by advertisement distributed in Bergen, in Hordaland, Norway. Only those that had no current somatic disease, no use of medication except hormonal birth control agents, and no history of psychiatric disorder were included. The healthy controls underwent the same baseline investigations as the ECT patient group, but did not receive ECT or anaesthesia.

ECT treatment

All patients received the standard ECT treatment as it is provided at the ECT-department at the Haukeland University Hospital in Bergen, Norway, administered with right unilateral electrode placement and a Thymatron System IV device (Somatics Inc., Venice, FL, USA), providing brief- or ultra-brief-pulse (0.25–0.5 ms), square wave, constant current (900 mA). Anaesthesia was obtained with the short acting anaesthetic thiopental. Muscle relaxation was obtained with succinylcholine (1 mg/kg). Three sessions per week were given until remission or until no further improvement of symptoms was expected, with a maximum of 20 sessions. The initial stimulus dose was determined based on age, and subsequent adjustments were made after each treatment based on electroencephalographic parameters such as seizure duration, δ-waves and postictal suppression, as well as reorientation time and clinical effect.

Assessments

Symptom intensity was measured with Montgomery and Åsberg Depression Rating Scale (MADRS) [32] by the treating clinician before and after completed ECT-series. Response was defined as a reduction of more than 50% in MADRS score over the treatment series, and remission as a MADRS score lower than 10 after ECT.

Blood samples

Venous blood samples were collected after at least 8 h of fasting at two time points for each patient: prior to treatment and one to two weeks after the completed ECT-series (median = 10 days, interquartile range = 6 days). For controls, samples were collected at baseline. The samples were centrifuged and the serum separated and stored at −80 °C until analysis. Serum concentrations of tryptophan and eight metabolites kynurenine (Kyn), 3-hydroxykynurenine (HK), kynurenic acid (KA), xanthurenic acid (XA), anthranilic acid (AA), 3-hydroxyanthranilic acid (HAA), quinolinic acid (QA) and picolinic acid (Pic), as well as riboflavin (vitamin B2) and pyridoxal 5′-phosphat (PLP, vitamin B6), inflammatory marker neopterin and the nicotine metabolite cotinine were measured by Bevital (www.bevital.no) using liquid chromatography-tandem mass spectrometry [33]. QA and Pic, as well as isotope labelled internal standards $^{2}H_3$-QA and $^{2}H_4$-Pic, were added to the published assay [34] by including the ion pairs 168.0/78.9, 124.2/78.0, 171.0/81.0, and 128.2/82.0, respectively. Within-day and between-day CVs were 4–7% for QA and Pic, precision data for the other biomarkers analysed by this assay can be found in previous publication [34]. The renal function marker creatinine was also measured at baseline for evaluation of renal function [34].

Statistical analyses

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 23.0 (IBM Corp., Armonk, New York) and RStudio version 1.1.383 [35] with core package *stats* and additional packages *Tidyverse* and *ggsignif*. Baseline clinical data for controls and patients were compared using chi-square test for categorical variables and Mann-Whitney *U* test for continuous variables. Baseline biochemical data were compared using linear regression for log-transformed variables both unadjusted and adjusted for smoking using log-transformed levels of cotinine. Changes in patients' serum concentrations from before to after treatment were analysed using Wilcoxon paired test. The same analyses were also performed for patients divided in subgroups based on ECT response and remission.

Results

Demographics and clinical characteristics

Out of the 30 patients recruited, three were excluded - one due to missing baseline blood sample and two due to high serum creatinine values (>120 μmol/L). The 27 remaining patients (15 female and 12 male) had a median age of 46.0 years while the 14 controls (8 female and 6 male ($p = 1.00$)) had a median age of 42.5 ($p = 0.57$). There were 5 (36%) smokers in the control group and 14 (52%) among the patients ($p = 0.51$). There was a significant difference in depression symptom load as measured with MADRS, with a median score of 1.0 for the controls and 34.0 for the patients ($p < 0.001$). Details on clinical characteristics and medication for patients are given in Table 1.

ECT treatment variables and symptom severity before and after treatment

Anaesthesia was given with a median of 3.88 (IQR = 1.88) mg thiopental per kg body weight. ECT was delivered with a median

Table 1
Clinical characteristics and medication.

	Total	*n* (%)[1]/ Median (IQR)[2]		Min.	Max.
Unipolar depression[1]	24	19	(79.2)		
Bipolar depression[1]	24	5	(20.8)		
Age at inclusion[2]	27	46	(21.0)	22	65
Age at debut of depressive symptoms[2]	26	20	(11.8)	10	60
Years since debut[2]	26	19.5	(25.3)	1	42
Number of depressive episodes[2]	20	3	(3.25)	1	50
Length in weeks of current depressive episode[2]	24	39	(44.2)	3	156
Psychotic symptoms in current depressive episode[1]	26	4	(15.4)		
Previous ECT treatment[1]	26	2	(7.69)		
No medication[1]	27	0	(0.00)		
Only litium[1]	27	1	(3.70)		
Only quetiapin[1]	27	2	(7.40)		
Two or more medications[1]	27	24	(88.8)		

Only patients were included (n = 27). Medication refers to the use of antidepressants, mood stabilisers and/or antipsychotics.Abbreviations: IQR, interquartile range.

charge of 237.8 mC (IQR = 134) and the median seizure length was recorded as 50.7 s (IQR = 16). The median MADRS score decreased from 34 pre-treatment to 15 post-treatment. Twelve patients responded to treatment (57.1%), whereas remission occurred in eight patients (38.1%). While the number of treatments did not differ between the 12 responders and 9 non-responders (10.3 and 12.1, respectively), there was a significant difference in the number of treatments between the 8 remitters and the 13 non-remitters (8.3 and 12.8, respectively, $p = 0.008$).

Tryptophan metabolites in patients and controls

The comparison of serum concentrations of tryptophan and metabolites for patients and the age and gender matched healthy controls are given in Table 2. In the unadjusted analyses, patients had significantly lower concentrations of KA, XA and Pic, as well as lower KA/Kyn, KA/QA, XA/HK and Pic/QA, while there were no statistical differences in measures of Trp, Kyn, HK, AA, HAA and QA or KTR between the groups. Adjusted for cotinine, KTR was higher while XA, KA/Kyn, KA/QA, XA/HK and Pic/QA were lower in the patient group compared to controls.

Changes in tryptophan metabolites in MDD patients after ECT

Post-treatment blood samples were available for 21 patients, of whom 12 responded to ECT while 9 did not. Wilcoxon analyses showed significant increase of HAA ($p = 0.028$), Pic ($p = 0.013$), Pic/QA ($p = 0{,}018$) and neopterin ($p < 0.001$) (Fig. 2, Supplementary Table 1). With patients divided in subgroups based on treatment response, there was significant increase in HK and Pic among

Table 2
Baseline concentrations and ratios or tryptophan metabolite and related metabolites in MDD patients compared to healthy controls.

	Baseline values				Linear regression			
	Control		Patient		Unadjusted		Adjusted for cotinine	
	(*n* = 14)		(*n* = 27)					
	Median (IQR)		Median (IQR)		Estimate	*p*-value	Estimate	*p*-value
Trp, μmol/L	77.2	(9.45)	75.2	(10.3)	−0.07	0.18	−0.09	0.11
Kyn, μmol/L	1.39	(0.47)	1.53	(0.42)	0.01	0.87	0.04	0.54
KA, nmol/L	45.3	(12.8)	37.4	(18.1)	−0.21	**0.04**	−0.16	0.11
HK, nmol/L	37.8	(7.77)	37.3	(13.3)	−0.07	0.46	−0.02	0.79
XA, nmol/L	15.9	(4.83)	10.2	(6.80)	−0.47	**0.00**	−0.44	**0.00**
AA, nmol/L	19.2	(9.15)	15.6	(6.75)	−0.11	0.26	−0.10	0.32
HAA, nmol/L	38.0	(13.6)	29.2	(12.6)	−0.20	0.10	−0.17	0.17
QA, nmol/L	318	(114)	329	(155)	0.09	0.38	0.14	0.15
Pic, nmol/L	32.9	(13.7)	25.2	(14.0)	−0.29	**0.01**	−0.28	0.02
KTR, ratio[a]	18.1	(4.07)	20.1	(5.62)	0.08	0.18	0.13	**0.02**
KA/Kyn, ratio[a]	31.9	(2.87)	26.1	(8.01)	−0.22	**0.00**	−0.20	**0.00**
KA/HK, ratio[b]	12.6	(1.97)	10.0	(3.20)	−0.14	0.16	−0.13	0.20
KA/QA, ratio[c]	15.2	(4.59)	10.9	(3.62)	−0.30	**0.00**	−0.30	**0.00**
XA/HK, ratio[c]	45.6	(10.8)	28.5	(14.4)	−0.40	**0.01**	−0.42	**0.00**
Pic/QA, ratio[a]	106	(37.3)	73.5	(31.3)	−0.38	**0.00**	−0.42	**0.00**
PLP, nmol/L	63.5	(14.4)	49.1	(34.9)	−0.19	0.21	−0.17	0.27
Riboflavin, nmol/L	11.9	(4.13)	14.6	(5.80)	0.07	0.54	0.13	0.23
Creatinine, μmol/L	71.8	(10.7)	73.8	(18.7)	0.04	0.42	0.06	0.25
Neopterin, nmol/L	14.3	(7.10)	17.7	(9.30)	0.23	0.07	0.29	0.02
Cotinine, nmol/L	0.49	(250)	298	(1120)	1.51	0.16		

Estimates and *p*-values from linear regression for log-transformed variables with and without adjustment for log-transformed cotinine. *p*-values below significance threshold 0.05 are marked in bold. Abbreviations: Trp, tryptophan; Kyn, kynurenine; HK, 3-hydroxykynurenine; KA, kynurenic acid; XA, xanthurenic acid; AA, anthranilic acid; HAA, 3-hydroxyanthranilic acid; QA, quinolinic acid; Pic, picolinic acid; PLP, pyridoxal 5′-phosphat; IQR, interquartile range. Ratios are multiplied by.

[a] 1000.
[b] 10 or.
[c] 100.

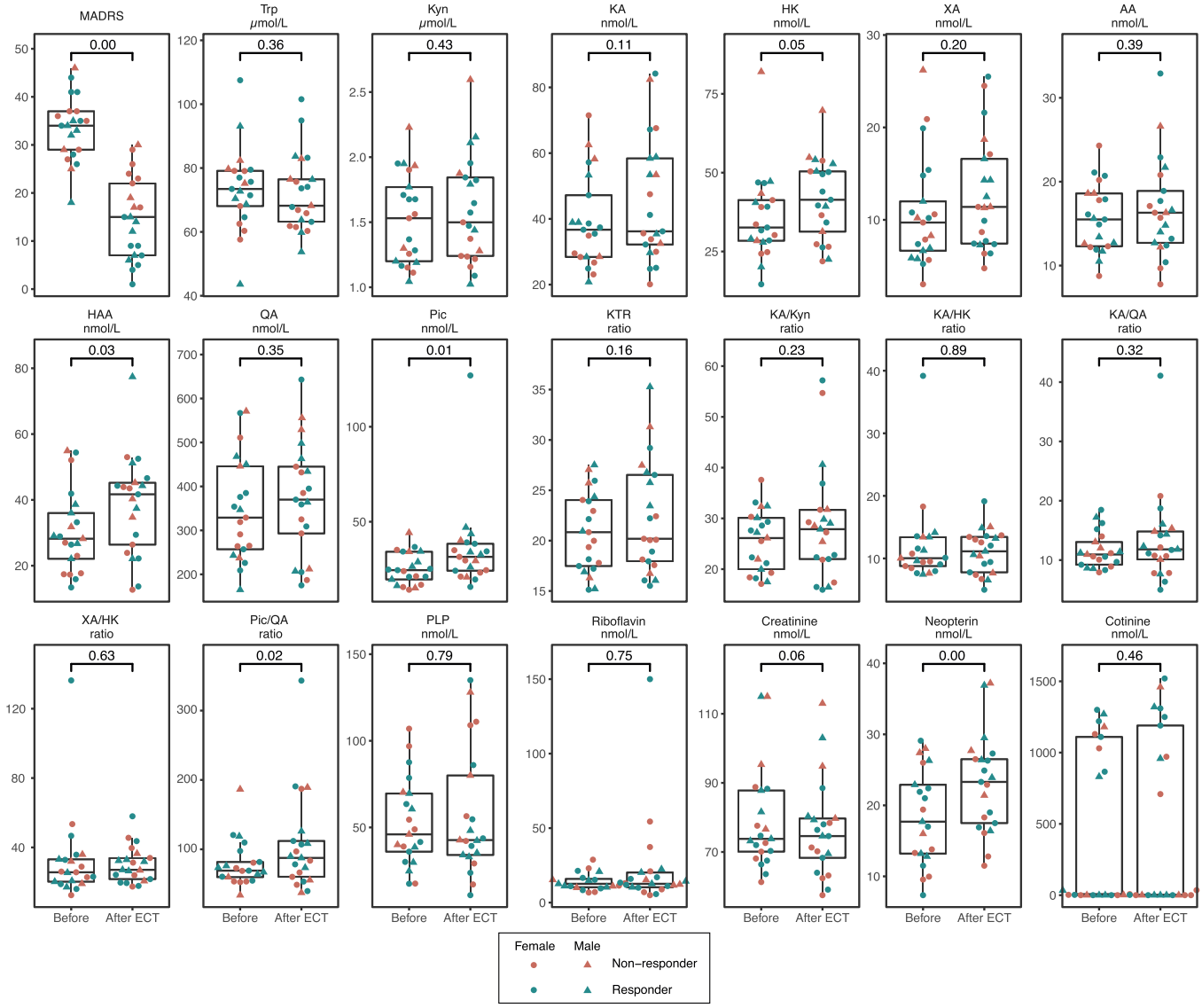

Fig. 2. MADRS and biomarker levels before and after ECT treatment. The horizontal box lines show the first (Q1), second (Q2) and third quartile (Q3). The whiskers cover all values between Q1 - 1.5 * IQR and Q3 + 1.5 * IQR. The *p*-value from Wilcoxon test of values before and after ECT is displayed for each variable. Y-axis scale is indicated below each variable's name. Abbreviations: MADRS, Montgomery and Åsberg Depression Rating Scale; Trp, tryptophan; Kyn, kynurenine; KA, kynurenic acid; HK, 3-hydroxykynurenine; XA, xanthurenic acid; AA, anthranilic acid; HAA, 3-hydroxyanthranilic acid; QA, quinolinic acid; Pic, picolinic acid; PLP, pyridoxal 5′-phosphat; Ribo, riboflavin; Creat, creatinine; Neopt; neopterin; Cot, cotinine.

responders (Table 3). There was also a significant increase in neopterin concentration both in responders and non-responders. Other metabolites concentrations and ratios remained unchanged. Analyses in remitters ($n = 8$) showed the same direction of effect as in patients with treatment response though no changes were significant. In the non-remitters ($n = 13$) there were significantly increased levels of neopterin (Supplementary Table 2).

Discussion

This study aimed to investigate serum concentrations of kynurenine metabolites in MD patients referred to ECT in comparison with healthy controls and to assess the effect of ECT on the kynurenine pathway. There were three main findings:

i) Compared to healthy controls, patients had low levels of kynurenine metabolites KA, XA and Pic and ratios KA/Kyn, KA/QA, XA/HK and Pic/QA, indicative of an imbalance in favour of neurotoxic substances.
ii) Comparing post-treatment to baseline concentrations, there was no reduction of KTR as a proxy measure for inflammation, nor in the concentration of inflammation marker neopterin. On the contrary, the concentration of neopterin was significantly increased after ECT.
iii) After treatment there was an increase in patient concentrations of HAA and Pic, metabolites with putative neuroprotective properties, and in the Pic to QA ratio.

Altered kynurenine pathway metabolism has been proposed as a link between mild chronic inflammation and depressive symptoms [5–7]. Inflammation can affect the kynurenine pathway both by activation of IDO, reflected by an increased KTR, and by activation of KMO, increasing metabolism through the HK branch of the pathway and causing an imbalance between neuroprotective and neurotoxic metabolites. In our study, like in a recent meta-analysis

Table 3
Changes in tryptophan metabolite concentrations and ratios after ECT treatment for responders and non-responders.

	Responders ($n = 12$)					Non-responders ($n = 9$)				
	Before ECT		After ECT		p-value	Before ECT		After ECT		p-value
	Median (IQR)		Median (IQR)			Median (IQR)		Median (IQR)		
MADRS, score	34.0	(5.50)	8.00	(6.75)	**0.00**	35.0	(8.00)	23.0	(7.00)	**0.01**
Trp, μmol/L	73.1	(7.67)	73.9	(19.7)	1.00	75.2	(16.6)	66.9	(13.8)	0.13
Kyn, μmol/L	1.52	(0.53)	1.72	(0.41)	0.08	1.53	(0.64)	1.28	(0.26)	0.65
KA, nmol/L	38.0	(7.25)	38.7	(26.9)	0.23	29.5	(29.8)	35.6	(20.9)	0.50
HK, nmol/L	32.2	(13.7)	42.7	(12.2)	**0.03**	33.6	(12.9)	36.5	(26.5)	0.57
XA, nmol/L	8.79	(6.24)	11.2	(7.46)	0.20	9.71	(2.73)	11.4	(8.42)	0.65
AA, nmol/L	15.2	(4.05)	15.7	(6.53)	0.17	17.8	(6.30)	16.3	(4.90)	0.73
HAA, nmol/L	29.0	(11.3)	43.0	(20.2)	0.06	27.0	(14.1)	40.3	(17.5)	0.36
QA, nmol/L	351	(163)	367	(158)	0.14	319	(181)	385	(152)	1.00
Pic, nmol/L	25.0	(9.10)	34.2	(14.6)	**0.03**	21.3	(18.6)	29.4	(8.30)	0.20
KTR, ratio[a]	19.6	(6.89)	22.8	(9.11)	0.09	20.8	(4.68)	20.1	(4.29)	1.00
KA/Kyn, ratio[a]	27.4	(8.64)	25.3	(11.0)	0.47	25.5	(11.0)	28.5	(6.28)	0.36
KA/HK, ratio[b]	10.7	(4.66)	10.9	(4.49)	0.85	9.51	(1.99)	12.6	(5.77)	0.65
KA/QA, ratio[c]	10.9	(6.39)	12.1	(4.51)	0.52	10.9	(1.95)	10.7	(4.73)	0.50
XA/HK, ratio[c]	24.3	(13.9)	26.8	(11.3)	0.91	26.1	(9.13)	31.2	(12.5)	0.57
Pic/QA, ratio[a]	7.48	(3.43)	9.84	(3.84)	0.06	5.99	(1.94)	6.65	(4.13)	0.25
PLP, nmol/L	40.1	(35.0)	40.9	(16.0)	0.47	48.9	(30.3)	56.5	(66.8)	0.73
Riboflavin, nmol/L	13.7	(5.25)	13.0	(6.90)	0.96	11.4	(5.32)	12.2	(12.1)	0.73
Creatinine, μmol/L	73.5	(13.6)	75.5	(10.4)	0.47	76.6	(18.7)	71.3	(16.5)	**0.03**
Neopterin, nmol/L	19.4	(9.43)	24.4	(8.00)	**0.03**	16.0	(12.7)	21.4	(10.4)	**0.01**
Cotinine, nmol/L	432	(1138)	480	(1265)	0.12	2.34	(1030)	0.00	(709)	0.55

Wilcoxon paired test. p-values below the significance treshold 0.05 are marked in bold. Only patients without missing data were included ($n = 21$). Abbreviations: MADRS, Montgomery and Åsberg Depression Rating Scale; Trp, tryptophan; Kyn, kynurenine; HK, 3-hydroxykynurenine; KA, kynurenic acid; XA, xanthurenic acid; AA, anthranilic acid; HAA, 3-hydroxyanthranilic acid; QA, quinolinic acid; Pic, picolinic acid; PLP, pyridoxal 5′-phosphat; IQR, interquartile range.Ratios are multiplied by.

[a] 1000.

[b] 10 or.

[c] 100.

[36], there was no significant difference in KTR between healthy controls and patients with MD in the unadjusted analyses. However, adjusted for cotinine, KTR was higher in patients, indicating increased INF-γ mediated activation of cellular immunity. Furthermore, there were lower levels of KA, XA and Pic, and lower KA/Kyn and KA/QA in patients compared to controls. This is in line with other studies on blood and CSF samples from depressed or suicidal patients showing an imbalance in the kynurenine pathway in favour of neurotoxic metabolites [6,14–17,30,37,38]. Comparing patients with healthy controls, these studies have shown higher levels of neurotoxic kynurenines [37,38], lower levels of neuroprotective kynurenines [6,17,30,38] and altered kynurenine ratios with lower KA/Kyn [6,17] and KA/QA [14–16]. However, one study found normal levels of kynurenines in depressed patients compared to healthy controls [39].

ECT has been found to elevate KTR in a study with 23 patients with MD [40]. Like two other studies assessing changes of KTR during ECT [16,17], we found no such change in KTR after treatment. However, after treatment we found significant increase in the patient concentrations of the inflammation marker neopterin, indicating an inflammatory response. Inflammation as response to ECT has been demonstrated in several studies [24]. Increased levels of proinflammatory cytokines have been observed as a short-term effect of single ECT sessions [25–27]. In our study, the post-treatment blood sample was drawn several days (median = 10 days, IQR = 6 days) after the last session in a series of ECT. Full series of ECT treatments like this have mostly been associated with a decrease in inflammation markers [27–29]. However, in a study by Hoekstra et al. a significant increase in neopterin serum concentration was detected in 20 severely depressed patients after ECT series [41]. Similarly, after a series of ECT, Freire and colleagues found increased levels of the proinflammatory cytokines TNF-α and INF-γ, both potent activators of kynurenine pathway enzymes IDO and KMO, although IL-6 concentration was reduced [42].

Previous studies on changes in the balance between neuroprotective and neurotoxic kynurenines after ECT have yielded inconsistent results: Schwieler and colleagues [16] reported a reduction in QA as well as in QA/KA in blood samples from 19 patients after ECT treatment. In addition to increased KTR, Guloksuz and colleagues found increased levels of KA, KA/Kyn and KA/HK after ECT [40]. In contrast, Olajossy and colleagues [30] found low levels of KA in pre-treatment blood samples of 50 patients across three diagnostic groups, but no significant increase in KA after treatment. Similarly, Allen and colleagues [17] found low plasma concentrations of KA and low KA/Kyn in patients before treatment, but no increase in KA after treatment, independent of response status. In the current study, only two kynurenine metabolites, HAA and Pic, were significantly increased after treatment. These metabolites both belong to the KMO branch of the kynurenine pathway starting with the KMO mediated conversion of Kyn to HK. It is interesting to note that both Pic and HAA are proposed as neuroprotective substances and that Pic is though of as an escape route preventing high levels of the neurotoxic QA (Fig. 1) [20,43,44].

In sum, it is possible that an ECT associated inflammation response has caused increased metabolism through KMO and the neurotoxic branch of the kynurenine pathway. KMO is stimulated by the same pro-inflammatory cytokines that cause activation of neopterin producing macrophages [9,10], and higher KMO activity could explain the observed increase in HAA and Pic.

To our knowledge this is the first study assessing a large panel of metabolites of the kynurenine pathway of tryptophan degradation and metabolite ratios reflecting enzymes involved in patients with MD before and after ECT treatment. The kynurenine pathway represents a potential mechanistic link between low-grade inflammation in depression and neuroplasticity. However, the small sample size, the lack of a control group of depressed patients not receiving ECT, and the complex contribution of the various kynurenine metabolites to the pathogenesis of depression, make it

difficult to distinguish the antidepressant mechanisms of action of ECT from other, nonspecific effects. Furthermore, there are important variables, such as systemic inflammation, nutrition, BMI and time of blood sampling, which could affect the tryptophan metabolism that we were unable to adjust for in this study. We excluded patients with renal failure, which may increase plasma concentration of metabolites with high renal clearance. Patients with somatic disorders other than renal failure were not excluded, and medications for somatic and psychiatric disorder may possibly affect concentrations of some metabolites. However, for each individual, medication was essentially stable during the study period, as only minor changes were done in drug therapy, mainly reduction of benzodiazepines and other substances raising seizure threshold. Compared to population-based studies [45], the response and remission rate in the current study are relatively low. This is probably due to a selection bias, as the included patients were younger and had a longer duration of the current episode, both factors known to be associated with lower response rates. The small sample size is a limitation of the study, as is the heterogeneous study population consisting of both bipolar and unipolar depression patients. The statistical power and the ability to detect "true" associations may be further reduced by normal variation in metabolite concentration over time [46], and such attenuations are likely because metabolite concentration was measured only at a single time point before and after ECT. However, the ability to detect biomarker status from a single measurement has been evaluated in terms of intraclass correlation constants (ICCs) for most kynurenine investigated, and ICCs varies in the range from 0.5 to 0.7 [47], which is considered as moderate to strong within-subject reproducibility [48].

In summary, the current study explored the impact of ECT on a large panel of kynurenine metabolites possibly involved in the pathogenesis of depression. The results from the current study are preliminary and should be followed up by studies in larger cohorts, also including a control group of depressed patients not receiving ECT. Future studies should also seek to measure a broader panel of inflammation markers and should ideally include measurements from cerebrospinal fluid (CSF). Furthermore, metabolites should be measured before start of treatment and after a predefined number of treatments, as well as at multiple time points after the final treatment.

Conclusion

Patients with major depression referred to ECT showed lower levels of neuroprotective kynurenine-pathway metabolites (KA, XA and Pic) as well as lowered neuroprotection ratios (KA/Kyn and KA/QA) compared to age- and sex-matched healthy controls. The results from this pilot study indicate that concentration of the inflammation marker neopterin was increased after ECT along with increased levels of Pic and HAA, two kynurenine metabolites with putative neuroprotective properties. Further research in larger cohorts is required to conclude whether ECT exerts its therapeutic effects via changes in the kynurenine pathway.

Authors' contributions

This study was designed and executed by UK, JH, TIMA, IL, VJE, LO and KØ. ØM, AU and PMU performed biochemical analyses. TIMA performed the statistical analyses and drafted the manuscript together with IL. All authors read and approved the final manuscript.

Declarations of interest

None.

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Abbreviations

AA	anthranilic acid
ACMSD	aminocarboxymuconate semialdehyde decarboxylase
ECT	electroconvulsive therapy; HAA, 3-hydroxyanthranilic acid
HK	3-hydroxykynurenine
IDO	indole 2,3-dioxygenase
KA	kynurenic acid
KAT	kynurenine aminotransferase
KMO	kynurenine 3-monooxygenase
Kyn	kynurenine
MADRS	Montgomery and Åsberg Depression Rating Scale
MD	major depression
NMDAr	*N*-methyl-D-aspartate receptor
PLP	pyridoxal 5′-phosphat; Pic, picolinic acid
QA	quinolinic acid
TDO	tryptophan 2,3-dioxygenase
Trp	tryptophan
XA	xanthurenic acid

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.brs.2019.05.018.

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