Circulating Endocannabinoids in Huntington’s Disease: An Exploratory Cross-Sectional Study

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Pre-press 29 January 2022

Abstract. Huntington’s disease (HD) is an inherited neurodegenerative disease characterized by motor, cognitive and behavioral deficits. Some evidence suggests that the endocannabinoid system participates in the pathophysiology of HD. We conducted a cross-sectional study comparing plasma levels of anandamide and 2-arachidonoylglycerol in manifest HD gene expansion carriers (HDGEC) and healthy controls, finding no difference in endocannabinoid levels between the groups. Correlations between endocannabinoid levels and clinical scales (Mini-Mental State Examination, Hospital Anxiety and Depression Scale, Unified Huntington Disease Rating Scale) were non-significant. We found a significant association between body mass index and anandamide levels in healthy controls but not in HDGEC.

Keywords: Huntington’s disease, endocannabinoid system, anandamide, 2-arachidonoylglycerol, clinical features, clinical scales

INTRODUCTION

Huntington’s disease (HD) is an inherited neurodegenerative disease characterized by motor, cognitive, and behavioral deficits often manifesting between 30 and 50 years of age [1]. The causative mutation is a CAG triplet repeat expansion in the huntingtin gene located on the short arm of chromosome 4. It is considered pathogenic when there are more than 35 CAG repeats [2]. HD gene expansion carriers (HDGEC) are clinically diagnosed with manifest HD when unequivocal clinical signs are present [3].

The endocannabinoid system may play a role in the pathophysiology of HD [2, 4]. Anandamide (AEA) and 2-arachidonoylglycerol (2AG) are the two main modulators of the endocannabinoid system, and there are two specific G-protein coupled cannabinoid receptors: type 1 (CB1) and type 2 (CB2) [5]. CB1 receptors are expressed primarily on the presynaptic terminals of excitatory and inhibitory neurons. CB2 receptors are found predominantly in the peripheral immune system but may also be present in the microglia [5]. The endocannabinoid system plays an
essential role in modulating basal ganglia, and CB1
receptors are highly expressed in these nuclei. In
HDGEC and animal models of HD, there is a marked
decrease of these receptors in the brain [6–8]. The
down-regulation of CB1 expression seems to occur
early in the disease and significantly affects the stri-
atal medium spiny neurons [9]. These abnormalities
seem to have a role in the pathophysiology of the dis-
ease. Furthermore, neuroprotective effects of some
cannabinoids in HD models reinforce the associa-
tion between the endocannabinoid system and HD
[4, 10, 11].

HD is known to have systemic features, such as
weight loss, altered glucose homeostasis, skeletal-
muscle atrophy, and blood cell abnormalities [12, 13,
24]. The endocannabinoid system is also widely dis-
tributed throughout the human body and is expressed
in several organs and systems with pleiotropic func-
tion, including regulation of metabolism and the
immune system [14]. A previous study reported
that fatty acid amide hydrolase activity was dra-
matically reduced in the peripheral lymphocytes of
HDGEC, leading to increased levels of AEA, since
it is metabolized by fatty acid amide hydrolase [15].
These observations may indicate that the peripheral
endocannabinoid system could also be compromised
in HDGECs. To our knowledge, no studies have
assessed endocannabinoid levels in the plasma or
serum of HDGECs. This study aimed to evaluate
possible abnormalities of peripheral circulating endo-
cannabinoids in HDGECs.

METHODS

We performed a cross-sectional study comparing
plasma levels of AEA and 2AG in HDGECs and
healthy controls. We invited all consecutive HDGECs
clinically and genetically diagnosed with HD who
were treated at the Movement Disorders Clinic of the
Ribeirão Preto Medical School Hospital, São Paulo,
Brazil between November 2016 and April 2018 to
participate. We recruited healthy controls from the
local community through local media announce-
ments. The healthy controls were not genotyped for
CAG expansion but were asked about their family
history of neurological diseases. We performed a
physical examination to rule out neurological abnor-
malities. The exclusion criteria for all participants
were: any significant or decompensated systemic
diseases (e.g., diabetes), diagnosis of other neuro-
logical diseases, active infectious or inflammatory
disease, pregnancy, or history of drug abuse. We
excluded healthy controls who were using antiepilep-
tics, antidepressants, or antipsychotics, as well as
those with any psychiatric disturbance (psychosis,
anxiety, or depression).

We collected demographic and clinical data from
all participants. All participants were assessed with
the Mini-Mental State Examination (MMSE) and the
healthy controls were also assessed with the Mini
International Neuropsychiatric Interview to exclude
psychiatric disorders. We evaluated HDGECs with
the Hospital Anxiety and Depression Scale (HADS)
and the Unified Huntington Disease Rating Scale
(UHDRS).

We collected peripheral blood samples by venous
puncture (approximately 15 mL per individual), be-
tween 8 am and 10 am, since the endocannabinoid
system may be affected by circadian rhythm [14]. The
blood was immediately centrifuged, and the plasma
was frozen at −70°C. We asked all participants to
fast for at least 8 h prior to blood collection, as well
as to avoid exercise and alcohol on the day before
collection.

The plasma levels of AEA and 2AG were mea-
sured using a previously validated column switching
method with bi-dimensional chromatography-tan-
dem mass spectrometry [16]. Blood analysis was
performed blindly, and each sample was measured
in triplicate. We also examined C-reactive protein
and glucose, total cholesterol, LDL cholesterol, HDL
cholesterol, triglycerides, and hepatic transaminase
levels.

We used the nonparametric Mann-Whitney test
for statistical analysis. We tested for sex differences
between the groups using the Z-score for two popu-
lation proportions. Correlations were analyzed with
Spearman’s correlation coefficient, and the Shapiro-
Wilk test was used to assess data distribution.

We estimated the sample size according to the
number of manifest HDGEC patients regularly seen
in our clinic (approximately 50). The local ethics
committee approved the study (HCRP-USP, num-
ber 1.828.036, November 2016/CAAE 59355416.3.
0000.5440). All participants provided written in-
formed consent.

RESULTS

We invited 50 manifest HDGECs to participate in
the study, but 6 declined. We excluded 2 because
it was impossible to collect complete clinical data
or blood samples. We evaluated 32 healthy controls
Table 1
Demographic and clinical features of HD gene expansion carriers (HDGEC) and healthy controls (HC). Values are represented as median (IQR), absolute numbers (n), or percentage (%)

<table>
<thead>
<tr>
<th>HDGEC</th>
<th>HC</th>
<th>p</th>
</tr>
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<tbody>
<tr>
<td>Number (n)</td>
<td>42</td>
<td>30</td>
</tr>
<tr>
<td>Female (%)</td>
<td>59.5%</td>
<td>56.6%</td>
</tr>
<tr>
<td>Age (y)</td>
<td>48.5 (43–58)</td>
<td>47.0 (39–54)</td>
</tr>
<tr>
<td>Education (y)</td>
<td>4.5 (4–10)</td>
<td>11.0 (11–15)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>61.0 (54.5–70)</td>
<td>69.5 (65.1–77.5)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>163 (155–168)</td>
<td>166 (160–170)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>22.7 (20.3–25.7)</td>
<td>24.9 (23.2–28.2)</td>
</tr>
<tr>
<td>MMSE</td>
<td>20.5 (16–23)</td>
<td>29.5 (29–30)</td>
</tr>
<tr>
<td>MMSE (z score)</td>
<td>–2.0 (–3; –0.6)</td>
<td>0.8 (0.4;1.2)</td>
</tr>
<tr>
<td>Antidepressant use (n)</td>
<td>32</td>
<td>0</td>
</tr>
<tr>
<td>Antipsychotic use (n)</td>
<td>34</td>
<td>0</td>
</tr>
<tr>
<td>Disease duration (y)</td>
<td>7.0 (4–10.4)</td>
<td>–</td>
</tr>
<tr>
<td>UHDRS motor score (0–124 pts)</td>
<td>37.5 (30.5–71)</td>
<td>–</td>
</tr>
<tr>
<td>Independence scale (10–100%)</td>
<td>70% (60–85)</td>
<td>–</td>
</tr>
</tbody>
</table>

HADS, Hospital Anxiety and Depression Scale; HADS-A, anxiety score; HADS-D, depression score; IQR, interquartile range; MMSE, Mini-Mental State Examination; UHDRS, Unified Huntington Disease Rating Scale; aZ-score test; bMann-Whitney test

and excluded 2 due to significant depressive symptoms. Table 1 describes the demographic and clinical features of all participants. Most variables were non-normally distributed.

The final sample included 42 manifest HDGECs and 30 healthy controls. The groups were matched for age and sex, and women predominated in both groups. Regarding disease stage, 19% of the HDGECs were Shoulson-Fahn stage I, 29% were stage II, 19% were stage III, and 33% were in the late stages (IV–V). The HDGEC group had a significantly lower education, weight, body mass index (BMI), and MMSE scores than the control group. Median disease duration in the HDGEC group was 7 years, and most used antidepressants and/or antipsychotics.

There was no difference between the groups regarding C-reactive protein, glucose, total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, and aminotransferase plasma levels. Figure 1 shows plasma endocannabinoid levels in the HD and control groups.

As presented in Fig. 1, there was no significant difference in endocannabinoid levels between the groups (AEA vs. AEA p = 0.11, 2AG p = 0.14). We found no difference between endocannabinoid levels in the HDGEC group, regardless of antidepressant or antipsychotic use (antidepressants vs. AEA p = 0.85; antidepressants vs. 2AG p = 0.54; antipsychotics vs. AEA p = 0.39; antipsychotics vs. 2AG p = 0.76). There was also no significant correlation between endocannabinoid levels and HADS scores (HADS vs. AEA p = 0.64, r = 0.07; HADS vs. 2AG p = 0.26, r = 0.17), endocannabinoid levels and MMSE scores (MMSE vs. AEA p = 0.98, r = 0.003; MMSE vs. 2AG p = 0.99, r = −0.002), or endocannabinoid levels and UHDRS motor scores (UHDRS vs. AEA p = 0.56, r = −0.08; UHDRS vs. 2AG p = 0.24, r = 0.17).

We found a significant correlation between BMI and AEA levels in healthy controls (p = 0.02), although the correlation coefficient was weak-to-moderate (r = 0.42). Other associations between BMI and endocannabinoid levels were non-significant (healthy control BMI vs. 2AG p = 0.83, r = 0.03; HD BMI vs. AEA p = 0.99, r = 0.0004; HD BMI vs. 2AG p = 0.28, r = 0.16).

Fig. 1. Plasma endocannabinoid levels (anandamide – AEA 2-arachidonoyl-glycerol – 2AG) of HD gene expansion carriers (HDGEC) and healthy controls (HC). Values are represented in ng/ml. **AEA p = 0.11, 2AG p = 0.14**.
DISCUSSION

In this study, we did not find altered levels of circulating endocannabinoids in HDGECs. In contrast, a previous study reported high levels of AEA in the peripheral lymphocytes of HDGECs due to reduced fatty acid amide hydrolase activity [15]. Multiple factors can affect circulating endocannabinoid levels, such as stress, exercise, inflammation, chronic pain, drug abuse, circadian rhythm, and food presentation and consumption [14]. AEA can also originate from multiple sources, such as blood cells, adipose tissue, muscles, the gastrointestinal tract, and the brain [14]. Several factors can contribute to changes in the blood levels of endocannabinoids, such as metabolic system dysregulation, immune system dysfunction, or altered production in the central nervous system (CNS). For this reason, circulating endocannabinoids may be less predictable and more subject to variation than measurements taken in the CNS or the immune system, which explains our findings.

We investigated whether antidepressants and depression could have modulated the plasma endocannabinoid levels of our patients but found no relationship between depressive symptoms or antidepressant use and endocannabinoid levels in HDGECs [23, 29].

Experimental studies in animal models of HD have shown low AEA and 2AG levels in basal ganglia and high AEA levels in the cerebral cortex, suggesting that endocannabinoid levels in the CNS could be altered in HD [21, 22]. Although altered endocannabinoid production in the CNS can reflect peripherally, the correlation between central and peripheral endocannabinoid levels is not predictable. A study that evaluated endocannabinoid levels in cannabis users and controls found no association between cerebrospinal fluid and serum levels of AEA or 2AG [25].

Peripheral mechanisms should also be considered when determining plasma endocannabinoid levels. One study found a strong positive association between adiposity and circulating AEA, but not with cerebrospinal fluid AEA levels [26].

We found a significant correlation between BMI and AEA levels in healthy controls but not in HDGECs. Nevertheless, it was a weak to moderate association ($r=0.42$), and we cannot rule out a false-positive result. Nevertheless, this finding could indicate subtle endocannabinoid system impairment in HDGEC regarding the control of energy metabolism or other metabolic routes. Previous studies have reported positive, but not always straightforward, correlations between endocannabinoids and BMI, and these were mainly observed in obese individuals, which was not a characteristic of our study participants. One study found an association between BMI and 2AG, but not AEA [27], while another reported an association between BMI and AEA, but not 2AG [28].

Our findings neither exclude nor confirm compartmentalized changes in endocannabinoid levels in some peripheral tissues, such as in the immune system [14]. The presented data is insufficient to draw definitive conclusions regarding the possible role of peripheral endocannabinoid system in the pathophysiology of HD.

Our study had several limitations. The small sample size undermined our statistical power. The lack of an endocannabinoid analysis in the CNS (e.g., cerebrospinal fluid study) limited comparison with circulating endocannabinoid levels. The existence of factors that could influence peripheral endocannabinoid levels can complicate the interpretation of plasma endocannabinoid levels [14].

CONFLICT OF INTEREST

The authors have no conflict of interest to report.

REFERENCES


