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Comparison of plasma, saliva, and hair lamotrigine concentrations

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ABSTRACT

Background: In some clinical situations (pregnancy, aging, drug resistance, toxicity), measurements of lamotrigine plasma levels may be reliable. Limited studies indicate that saliva and hair could be alternative sources for monitoring lamotrigine therapy. The drug content in hair can also be used to assess the history of drug therapy and to ascertain long-term patient compliance. The aims of this study were to 1) determine the correlations among plasma, saliva, and hair lamotrigine concentrations, 2) evaluate saliva as an alternative matrix for monitoring drug levels and 3) evaluate hair as a source of information on adherence to antiepileptic treatment and on the correlation of hair concentrations with clinical outcomes in patients with epilepsy.

Methods: Plasma, saliva, and hair lamotrigine concentrations were measured by liquid chromatography–tandem mass spectrometry in positive ionization mode. The study group (n = 85) was recruited among the epileptic patients at the Institute of Psychiatry and Neurology, Warsaw, Poland.

Results: Plasma concentrations were not influenced by sex, age, or the concomitant use of other antiepileptic drugs. Lamotrigine saliva and plasma concentrations were strongly correlated ($r = 0.82$, $p < 0.001$). Lamotrigine hair concentrations were correlated with the plasma concentrations ($r = 0.53$, $p < 0.001$) and daily dose in mg/kg ($r = 0.23$, $p = 0.024$). The analysis revealed no significant correlation between lamotrigine hair levels and the number of seizures in the previous 3 months ($r = -0.1$, $p > 0.05$).

Conclusions: The lamotrigine saliva concentration is strongly correlated with its plasma level, and saliva can be used as an alternative matrix to plasma for monitoring. Lamotrigine can also be successfully measured in hair, and the drug levels in hair tend to be correlated with the levels in plasma. However, lamotrigine levels in hair may not correspond to clinical outcomes (i.e., seizure episodes).

1. Introduction

Lamotrigine (LTG) is a commonly used antiepileptic drug, indicated for the treatment of epilepsy and several other medical conditions, i.e., maintenance treatment of bipolar disorder, neuropathic pain, and depression and as an add-on therapy to neuroleptics and antidepressants [1–6]. The literature findings are inconclusive with regard to the relationship between the LTG level and its efficacy and toxicity [7,8]. Concentration monitoring is useful to assess patient compliance and the influence of concomitant therapy, pregnancy, aging, renal or hepatic failure and other factors on drug pharmacokinetics. For example, pregnancy or oral contraceptive use may reduce LTG concentrations by more than 50% [9–11].

Measurement of drug concentrations in saliva seems to be a reliable method for monitoring LTG exposure [12–17]. The LTG concentration in saliva strongly correlates with the concentration in plasma. The advantages of saliva sampling include noninvasiveness, repeatability, and simplicity. In addition, the salivary drug concentration reflects a pool of free, unbound drugs in the blood, which better corresponds to the biological activity of the drug.

In addition to saliva, hair is another easily accessible and interesting biological matrix for testing drug concentrations. The root of every growing hair is constantly exposed to any drug that is circulating in the blood [15]. Hair can be used to record the history of drug exposure and ascertain variable and intermittent compliance. Hence, the assessment of drug contents in hair may possibly be used to monitor the history of

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Table 1
Subject characteristics and individual plasma, saliva, and hair lamotrigine (LTG) concentrations.

Patient No.	Sex	Age, years	LTG Daily Dose, mg	LTG Daily Dose, mg/kg	LTG Plasma Level, mg/L	LTG Saliva Level, mg/L	LTG Saliva/ Plasma Ratio	LTG Hair Level, mcg/mg	No. of Seizure Episodes in the Previous 3 Months
1	M	21	400	4.60	8.64	1.32	0.15	–	0
2	F	28	350	5.38	14.03	13.68	0.98	–	12
3	F	32	300	4.92	6.74	4.17	0.62	0.27	0
4	M	29	250	3.85	6.39	3.89	0.61	0.18	27
5	F	27	300	5.36	4.96	2.68	0.54	0.05	20
6	M	44	450	6.62	**15.36**	14.70	0.96	3.62	17
7	F	51	350	3.89	8.47	3.83	0.45	0.76	8
8	F	20	350	3.89	9.03	2.91	0.32	0.56	15
9	F	29	250	4.31	13.98	5.02	0.36	0.27	20
10	F	31	400	3.88	2.93	0.81	0.28	1.09	12
11	F	50	300	1.44	4.43	0.83	0.19	0.46	11
12	F	19	400	5.19	5.01	1.31	0.26	0.16	2
13	M	48	200	2.86	5.36	4.28	0.80	0.42	30
14	F	33	150	3.06	2.53	0.62	0.25	0.07	0
15	M	30	225	2.23	6.66	4.96	0.74	0.57	2
16	F	20	300	5.45	4.32	4.83	1.12	1.80	40
17	F	63	300	2.40	1.73	1.01	0.58	0.19	1
18	M	59	200	2.56	1.86	0.99	0.53	0.14	6
19	F	31	250	3.73	2.29	1.11	0.48	0.08	15
20	F	34	200	2.99	1.83	2.21	1.21	0.17	1
21	M	45	200	2.22	2.03	1.16	0.57	0.60	10
22	M	39	300	3.33	7.74	1.51	0.19	1.98	0
23	M	42	400	4.44	**0.63**	0.49	0.78	0.14	4
24	F	61	200	2.15	1.82	1.26	0.69	0.41	3
25	M	27	125	1.74	2.15	1.30	0.61	0.11	1
26	M	67	100	1.32	1.87	1.31	0.70	0.18	0
27	F	38	200	3.23	2.57	0.80	0.31	0.21	1
28	F	43	250	4.31	3.90	2.97	0.76	0.15	14
29	F	25	250	4.10	6.33	3.48	0.55	0.29	0
30	F	29	450	7.76	10.4	6.18	0.59	0.19	1
31	M	50	100	1.23	**1.38**	1.30	0.94	0.05	7
32	M	50	225	1.73	1.60	0.92	0.57	0.88	3
33	F	27	200	3.17	5.89	2.89	0.49	–	0
34	F	58	150	3.49	3.54	2.92	0.83	0.12	9
35	M	21	125	1.92	2.62	1.61	0.62	0.09	0
36	M	23	300	3.95	7.46	2.69	0.36	0.51	43
37	F	54	225	4.59	4.49	2.99	0.67	0.05	2
38	F	24	300	4.23	4.02	2.75	0.68	0.91	0
39	M	17	150	1.60	1.96	1.02	0.52	0.13	0
40	M	41	100	1.43	5.14	3.39	0.66	0.11	0
41	F	62	150	2.14	1.68	1.06	0.63	0.11	0
42	F	56	100	0.91	**1.17**	0.50	0.34	0.05	10
43	F	26	200	4.08	3.37	2.20	0.65	0.05	13
44	M	21	250	3.85	3.17	2.10	0.66	0.05	11
45	F	27	400	6.78	1.94	1.24	0.64	0.05	0
46	F	44	50	0.68	1.58	0.98	0.62	0.15	0
47	M	42	150	2.50	4.19	4.53	1.08	0.16	10
48	F	26	150	2.42	2.99	2.06	0.69	0.05	110
49	F	37	150	2.11	3.47	1.69	0.49	0.08	90
50	F	24	25	0.50	**1.01**	0.55	0.54	–	6
51	M	61	200	2.20	1.54	1.20	0.78	0.05	31
52	F	44	350	4.67	10.05	9.43	0.94	0.19	90
53	F	37	300	4.92	3.60	2.79	0.78	0.05	0
54	F	27	100	2.00	2.07	1.75	0.85	0.05	0
55	F	19	300	7.32	7.32	3.88	0.53	0.50	10
56	F	77	250	2.98	1.67	1.32	0.79	0.05	3
57	F	41	200	2.70	**1.40**	0.57	0.41	0.15	0
58	F	21	200	3.33	3.60	1.61	0.45	0.10	4
59	F	39	150	1.67	2.68	1.57	0.59	0.18	0
60	M	30	225	2.23	2.83	2.76	0.98	0.51	11
61	F	20	300	5.45	6.09	4.26	0.7	0.88	40
62	F	43	250	4.31	**1.30**	0.46	0.34	0.09	14
63	F	58	150	3.49	2.33	2.33	1.00	0.10	9
64	F	54	225	4.59	1.75	0.75	0.43	0.50	19
65	M	41	100	1.43	**1.40**	1.23	0.88	0.13	0
66	F	41	200	3.92	3.62	3.95	1.09	0.22	1
67	F	27	100	2.00	1.89	1.78	0.41	0.17	0
Mean (SD)	M: 22; F: 45	37.69 −14.25	231.59 −98.41	3.37 −1.60	4.24 −3.26	2.64 −2.61	0.63 −0.24	0.36 −0.56	12.22 −21.27

– did not agree to have hair cut.
*Bold data indicate LTG plasma concentrations below or above the therapeutic range (1.5–14 mg/L).

antiepileptic drug therapy and to ascertain long-term patient compliance [18].

The concentration of LTG in hair and its correlation with the concentration in plasma and daily dose have not been studied. Recently, we published a study on the levetiracetam concentration in plasma, saliva and hair [19], which revealed that the levetiracetam concentration in saliva strongly correlates with its plasma level (r = 0.93) but the correlation between plasma and hair levels was much weaker (r = 0.36). These observations suggest that the levetiracetam hair content may reflect different aspects of chronic levetiracetam therapy than its plasma and saliva concentrations. It can be hypothesized that the drug hair concentration is related to chronic drug exposure and can be considered a biomarker of long-term patient compliance. For example, Ferrari et al. [20] recently showed that hair was a unique matrix with which to document chronic drug use in headache patients. The level of each individual drug in hair was a reliable marker of adherence to pharmacological treatment. The above considerations indicate that it will be interesting to determine how the LTG content in hair correlates with its plasma level, clinical outcomes and compliance. The purpose of the present study was to 1) determine correlations between the plasma, saliva, and hair LTG concentrations in epileptic patients, 2) evaluate saliva as an alternative matrix for monitoring drug levels, and 3) evaluate hair as a source of information on adherence to antiepileptic treatment and the correlation of the hair concentration with clinical outcomes in patients with epilepsy.

2. Materials and methods

Saliva, plasma, and hair samples were collected and assayed for LTG in relation to the daily dose of medication calculated in terms of mg per kg per day.

2.1. Chemicals and materials

LTG (purity ≥98%) and fluconazole (internal standard (IS), purity ≥98%), methanol (LC-MS-grade), acetonitrile (LC-MS-grade), formic acid (LC-MS-grade), and water (LC-MS-grade) were purchased from Sigma-Aldrich (Steinheim, Germany).

Venous whole blood samples were collected into EDTA blood collection tubes. Plasma was separated from blood cells by centrifugation (4 °C, 1000*g*) within 30 min of sampling and then frozen at −20 °C until analysis.

Saliva and hair were collected within 5 to 10 min of blood sampling. Unstimulated saliva samples were collected with commercially available Salivette devices (Sarstedt, Nümbrecht, Germany). All the saliva samples were centrifuged at 1000*g* for 2 min at room temperature and frozen at −20 °C until analysis.

Hair samples were collected in accordance with the recommendations of the Society of Hair Testing [21]. Hair samples were cut from the occipital head area as close as possible to the skin and then wrapped in aluminum foil such that the root end of the sample could be identified. The hair samples were stored at room temperature in sealable polyethylene bags until analysis of the LTG content.

2.2. Sample preparation

Plasma and saliva samples were thawed to room temperature. A 15 μL plasma/saliva aliquot and 100 μL of IS (1.14 mg/L) were transferred into 1.5-mL polypropylene microcentrifuge tubes. After vortexing for 30 s at 1500 rpm, 1000 μL of ethyl acetate was added. Samples were vortexed for another 2 min at 1500 rpm and then centrifuged for 5 min at 3000 rpm. The 500 μL organic layer was transferred into a clean polypropylene tube and evaporated to dryness at 40 °C using a gentle stream of nitrogen (Stuart sample concentrator, SBH CONC/1). The dry residue was reconstituted in 800 μL of the mobile phase by shaking on a Vibrax mixer for 10 min at 1900 rpm and transferred to an autosampler vial; 1.0 μL was injected into the liquid chromatography–tandem mass spectrometry (LC-MS/MS) system.

A single hair segment (1 cm) was cut from the hair root along the length of the individuals' hair. The 1-cm segment was fragmented into small pieces, and a 20 mg portion was transferred to a tube and digested by incubation in 1 mL of methanol at 60 °C for 1 h. After cooling, 50 μL of the supernatant and 100 μL of IS (1.14 mg/L) were transferred into a 1.5-mL polypropylene microcentrifuge tube. After vortexing for 30 s at 1500 rpm, 1000 μL of ethyl acetate was added. Samples were vortexed for an additional 2 min at 1500 rpm and then centrifuged for 5 min at 3000 rpm. The 500 μL organic layer was transferred into a clean polypropylene tube and evaporated to dryness at 40 °C using a gentle stream of nitrogen. The dry residue was reconstituted in 800 μL of the mobile phase by shaking on a Vibrax mixer for 10 min at 1900 rpm and transferred to the autosampler vessel; 1.0 μL was injected into the LC-MS/MS system.

2.3. Sample analysis

Plasma, saliva and hair samples were analyzed by an LC system (Shimadzu, Duisburg, Germany) equipped with two LC-20AD pumps, an SIL-20ACXR autosampler, a CTO-20AC column oven and a DGU-20A3 degasser. MS detection was performed on a tandem mass spectrometer (LCMS-8030, Shimadzu, Duisburg, Germany). Data processing was performed using the LabSolutions LCMS 5.42 SP3 software package for quantification (Shimadzu). A Kinetex analytical column (5 μm C18 100A, 100 × 2,1 mm) was used (Phenomenex, Torrance, CA, USA) with the corresponding guard column (Security Guard Ultra UHPLC 2.1 ID; Phenomenex, Torrance, CA, USA). The LTG concentration was quantified by ESI-MS/MS detection in positive-ion mode. Nitrogen was used as the nebulizing gas, and argon was used as the collision gas. The instrument settings were as follows: capillary voltage, 4 kV; N_2 nebulizer gas flow, 2.0 l/min; N_2 drying gas flow, 10.0 l/min; DL temperature, 200 °C; heat block temperature, 300 °C; and collision energy offset, −20.0 V for LTG and −10.0 V for IS (fluconazole). The temperature of the column compartment was set to 40 °C, and the needle rinse solvent was methanol. The mobile phase was an isocratic solvent system consisting of methanol, acetonitrile, water and formic acid in a ratio of 88:3:9:0.1 (v/v/v/v), and the flow rate was 0.3 mL/min. The total run time was 2 min, and the elution of LTG and the IS occurred at 0.59 and 0.72 min, respectively.

Samples were extracted by a simple liquid-liquid extraction method. Sample analysis was performed in multiple reaction monitoring mode (296.90 → 256.15 for LTG; 307.10 → 220.20 for the IS).

2.4. Study group

Eighty-five epileptic patients at the Institute of Psychiatry and Neurology, Warsaw, Poland, were enrolled between June 2014 and March 2017. Patients gave their written informed consent to participate in the study; all the procedures were approved by the local bioethics committee. Epilepsy was diagnosed in accordance with the International Statistical Classification of Diseases and Related Health Problems (ICD-10) [22]. Patients were examined by experienced neurologists using a structured interview that included gathering demographic and clinical data (etiology of epilepsy, course of treatment, current medication, concomitant disorders). All patients were taking LTG, and some (n = 39) were also treated with other antiepileptic medications (valproic acid, carbamazepine, tiagabine, clonazepam, oxazepam, topiramate, lacosamide, gabapentin). Patients were required to have had a history of ≥3 months of stable drug dosing. The effectiveness of the treatment was based on a seizure diary covering the previous 3 months. The adherence to LTG treatment was assessed by a neurologist using the Medication Adherence Questionnaire (MAQ) [23]. Prior to sampling, the patient had to have been fasted for at least 10 h (overnight fasting). The blood and saliva collection took place just

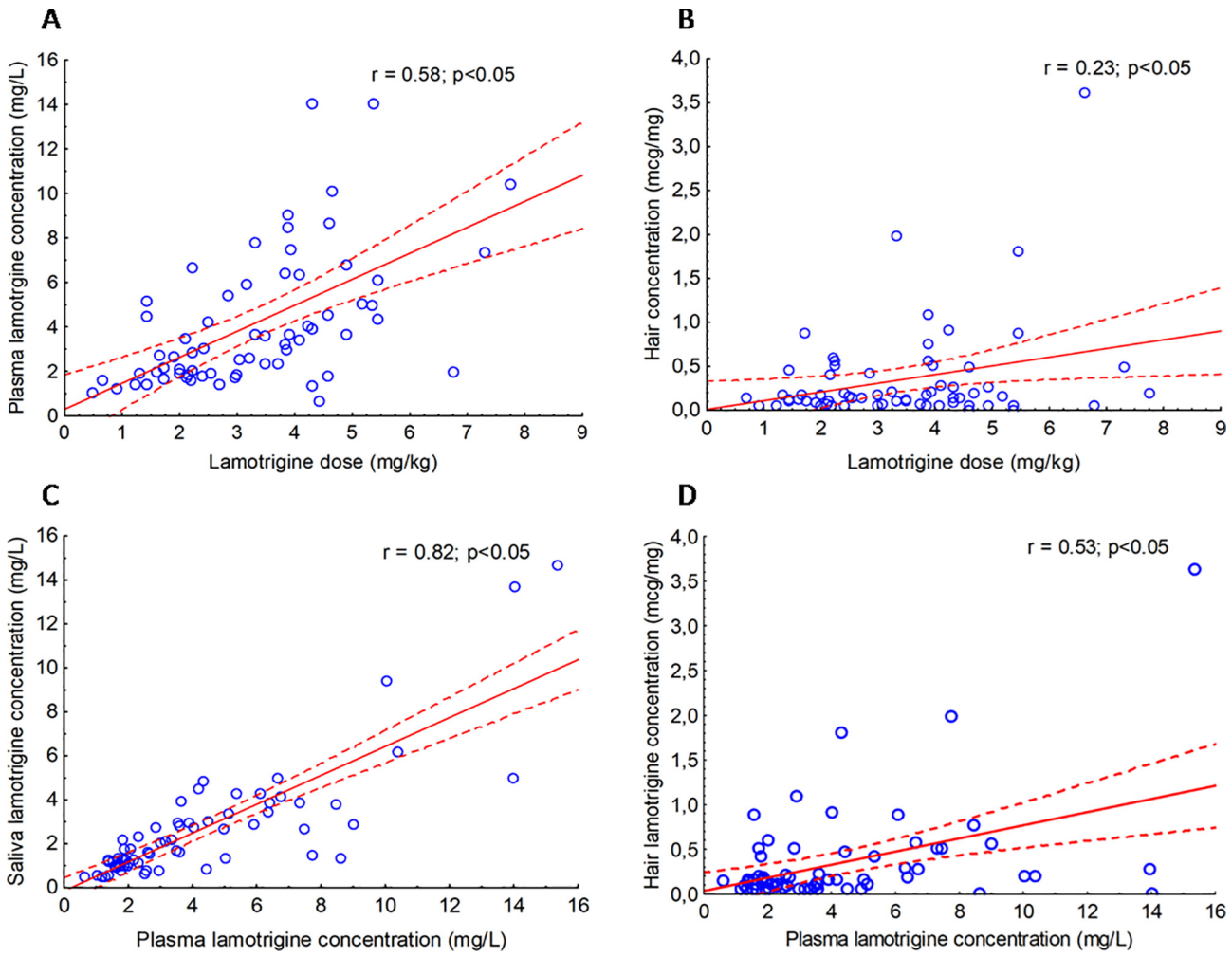

Fig. 1. Correlations between (A) Daily LTG dose (mg/kg) and plasma concentration (B) Daily LTG dose (mg/kg) and hair concentration (C) LTG concentrations in plasma and saliva (D) LTG concentrations in plasma and hair.

before the morning dose.

2.5. Method validation

All laboratory work was performed in compliance with the Good Laboratory Practice principles [24]. All validation experiments were performed according to the European Medicines Agency guidelines on the validation of bioanalytical methods [25].

2.6. Statistical analysis

All data were analyzed with the aid of the Statistica v.12 software package (StatSoft, Tulsa, OK). Relationships between the LTG concentration in different matrices were assessed from Pearson coefficients. Student's *t*-test was used to compare plasma concentrations between subgroups of patients. One-way analysis of variance (ANOVA) was used to assess the influence of hair color on the drug hair content. *P* values < 0.05 were considered significant.

3. Results

3.1. Method validation

3.1.1. Calibration curve, accuracy, and precision

Method validation was performed via analysis of samples spiked with a LTG standard. The calibration curve was linear over a range of 0.5–20 mg/L in plasma, 0.5–20 mg/L in saliva, and 0.05–2 mcg/mg in hair. The limits of quantification (LOQs) were 0.5 mg/L in plasma and saliva and 0.05 mcg/mg in hair. The correlation coefficients of the calibration curves were greater than 0.998.

3.1.2. Selectivity

In the chromatograms of 6 lots of blank plasma samples or saliva samples, no interfering peaks from endogenous substances were observed at the retention time of LTG.

3.1.3. Matrix effect

A matrix effect was not observed, as there was no significant ion suppression or enhancement of the analyte peak caused by coeluting compounds from the biological matrix (plasma, saliva, and hair).

3.1.4. Recovery

The mean extraction recovery for LTG in plasma was 99.77% at the low concentration level, 99.44% at the intermediate concentration level, and 101.20% at the high concentration level; that in saliva was 101.10%, 101.22%, and 101.22%, respectively; and that in hair was 100.52%, 99.19%, and 100.16%, respectively.

3.1.5. Stability

Analyte stability was assessed in quality control (QC) samples at 3 concentration levels with 3 replicates for each concentration. Short-term and long-term stability (3 months at −70 °C) assays showed no

Table 2
Studies investigating the LTG concentration in saliva and plasma.

	Conditions	Correlation between dose (mg/kg/day) and plasma level	Saliva/plasma ratio	Correlation between saliva and plasma
Our study	No of patients = 67; unstimulated saliva; LC-MS/MS*	r = 0.58	0.63	r = 0.82
Tmavska et al. (1991) [47]	No. of patients = 8; HPLC*	–	0.56	r = 0.95
Tsiropoulos et al. (2000) [17]	No. of patients = 40; unstimulated saliva; HPLC*	r = 0.47	0.55	r = 0.85
Tsiropoulos et al. (2000) [17]	No. of patients = 40; stimulated saliva; HPLC*	–	0.47	r = 0.94
Ryan et al. (2003) [16]	No. of patients = 31; HPLC*	–	0.62	r = 0.81
Malone et al. (2006) [14]	No. patients = 20; unstimulated saliva; HPLC*	–	0.48	r = 0.98
Malone et al. (2006) [14]	No. patients = 20; stimulated saliva; HPLC*	–	0.47	r = 0.98
Mallayasamy et al. (2010) [13]	No. patients = 23; HPLC*	–	–	r = 0.68
Devulder (2006) [46]	No. patients = 7; HPLC*	r = 0.95	–	–

* Method of LTG determination in saliva and plasma; LC-MS/MS – liquid chromatography–tandem mass spectrometry; HPLC – high-performance liquid chromatography.

significant change in the LTG concentration, as indicated by the mean recovery. Similarly, freeze-thaw cycles had no effect on LTG concentrations. Samples were stable for 24 h at 4 °C (postpreparative stability).

3.2. Clinical data

Eighty-five patients were recruited to the study, and 18 patients were excluded from the analysis because their LTG levels were below the LOQ in one of the analyzed matrices (plasma, saliva, hair). The final study group included 67 patients (21 inpatients, 46 outpatients; 22 men, 45 women; mean age: 37.7 ± 14.3 years). The patient characteristics and analysis results are presented in Table 1.

The mean (± SD) daily dose of LTG was 231.3 ± 98.4 mg (range: 25–450 mg). In relation to body weight, the mean daily dose was 3.4 ± 1.6 mg/kg. The mean LTG concentration was 4.2 ± 3.3 mg/L in plasma, 2.6 ± 2.6 mg/L in saliva, and 0.47 ± 1.1 μg/mg in hair. The mean plasma/saliva ratio was 1.9 ± 1.1.

There was a significant positive correlation between the LTG dosage in mg/kg/day and LTG plasma concentration ($r = 0.57$; $p < 0.001$; Fig. 1A).

The correlation between the LTG dose and hair content was weak but still statistically significant ($r = 0.23$, $p = 0.024$; Fig. 1B).

There was a strong and significant correlation between the LTG plasma and saliva levels ($r = 0.82$, $p < 0.001$; Fig. 1C) and between the plasma and hair levels ($r = 0.53$, $p < 0.001$; Fig. 1D).

Potential confounders, such as taking another antiepileptic drug ($t = 0.67$, $p > 0.05$), sex ($t = 0.1$, $p > 0.05$) or age ($r = -0.297$, $p > 0.05$), had no significant effect on LTG levels. One-way ANOVA revealed no effect of hair color (blonde, dark blonde, black, brown, gray) [$F_{(3,57)} = 0.23$, $p > 0.05$]. To the best of our knowledge, the LTG concentration in hair has not yet been studied, and only a few studies have evaluated the therapeutic range for LTG in serum [26–31].

There was no significant correlation between LTG hair levels and the number of seizures in the previous 3 months ($r = -0.1$, $p > 0.05$).

4. Discussion

To the best of our knowledge, this is the first study showing correlations of the LTG levels in plasma, saliva and hair with retrospective seizure control. The results of this study provide important information for clinical practice. First, LTG can be measured in both saliva and hair, and its levels these matrices correspond to the plasma content. Second, the LTG hair content correlates with the plasma concentration ($r = 0.53$, $p < 0.001$), which may reflect the history of drug exposure and good patient compliance [18,32]. Third, LTG hair levels are not associated with seizure control.

In general, our results are in line with those described previously by others (see Table 2).

In comparison to that observed by Devulder [46] or Krasniqi et al. [5], we observed a weaker correlation between the LTG plasma level and the daily dose taken ($r = 0.95$ or $r = 0.83$ vs $r = 0.57$, respectively). This may be partially explained by methodological differences: we measured the correlation in relation to the dosage in mg/kg/day, whereas the others used the daily dose in mg. Moreover, our group of patients ($n = 67$) was more than two times larger than the groups in the two other studies ($n = 7$ and $n = 30$, respectively).

Our study indicated that saliva is a reliable alternative matrix to plasma. The correlation between plasma and saliva drug concentrations was high ($r = 0.82$) and concurs with previous reports. Using saliva over plasma has several advantages. One is that the collection of saliva is simple and noninvasive, and the collection procedure is less expensive and more convenient than a blood draw. On the other hand, potential contamination, insufficient volume and difficulty in pipetting due to the viscosity are disadvantages of using saliva. The rationale for using saliva for drug monitoring is based on the assumption that the

diffusion of the drug into the saliva is proportional to the salivary flow rate. This seems to be reasonable for drugs with high (> 8.5) or low (< 5.5) pKa values because in this pKa range, the unbound protein fraction remains stable irrespective of changes in saliva pH [17]. LTG has a pKa of 5.5, and its saliva concentration significantly differs between stimulated and unstimulated saliva [17]. It is recommended that a standardized saliva collection technique be used in clinical practice.

Our previous studies with another antiepileptic drug, levetiracetam, showed only a weak correlation (r = 0.22) between the plasma and hair concentrations [19]. The reason for the stronger association between the hair and plasma drug contents in LTG-treated patients than in levetiracetam-receiving patients is interesting. One of the most obvious explanations may be that patients taking LTG have better compliance with the treatment. Drug hair concentrations may correspond with chronic drug exposure and can be considered a biomarker of long-term patient compliance [18,31–34]. Mei and Williams [35] found that measurement of hair concentrations is one of the most advanced methods of assessing compliance in epileptic patients. Williams et al. [18,36] found that the use of hair samples was more accurate than self-evaluation methods or plasma concentration monitoring. Assuming that hair levels correspond to patient compliance, we can conclude that patients taking LTG follow their recommendations better than do patients receiving levetiracetam.

Retrospective analysis revealed no association between LTG hair concentrations and seizure control in the previous 3 months (r = −0.1, p > 0.05). Only some single studies showed a significant correlation between serum concentration and efficacy [37,38], and most studies did not confirm such an association [39–42]. One important explanation may be that higher daily doses and higher concentrations typically relate to refractory patients with poor seizure control; thus, no correlation can be found. Other possible reasons for the lack of correlations include interindividual variability of epilepsy and other clinical and nonclinical factors that could affect the number of seizures.

Our study does not provide reasons to monitor the LTG concentration in hair to predict clinical outcomes. However, some authors claim that monitoring the concentration of LTG may play an important role in the treatment of refractory epilepsy and for treatment during pregnancy, as well as in chronic pain treatment [43–47]. It may also be reasonable to monitor drug levels to confirm suspected toxicity or when administered with an inhibitor or inducer of hepatic enzymes [26].

5. Conclusions

The LTG saliva concentration is highly correlated with its plasma level, and saliva can be used as an alternative matrix to plasma for LTG concentration measurements. LTG can also be measured in hair, which reflects its levels in plasma; however, the level in hair does not correspond to clinical outcomes.

Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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