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Salivary serotonin does not correlate with central serotonin turnover in adult phenylketonuria (PKU) patients

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ABSTRACT

Introduction: Phenylketonuria (PKU) is an inborn error of metabolism associated with an increased risk of behavioural and mood disorders. There are currently no reliable markers for monitoring mood in PKU. The purpose of this study was to evaluate salivary serotonin as a possible non-invasive marker of long-term mood symptoms and central serotonin activity in patients with PKU.

Methods: 20 patients were recruited from our Adult Metabolic Diseases Clinic. Age, sex, plasma phenylalanine (Phe) level, DASS (Depression Anxiety Stress Scales) depression score, DASS anxiety score, BMI, salivary serotonin, salivary cortisol, 2-year average Phe, 2-year average tyrosine (Tyr), and 2-year average Phe:Tyr ratio were collected for each patient. Spearman's ρ correlation analysis was used to determine if there was any relationship between any of the parameters.

Results: There were positive correlations between DASS anxiety and DASS depression scores (Spearman's $\rho = 0.8708$, p-value < 0.0001), BMI and plasma Phe level (Spearman's $\rho = 0.6228$, p-value = .0034), and 2-year average Phe and BMI (Spearman's $\rho = 0.5448$, p-value = .0130). There was also a negative correlation between salivary cortisol and plasma Phe level (Spearman's $\rho = -0.5018$, p-value = .0338). All other correlations were not statistically significant.

Conclusion: Salivary serotonin does not correlate with peripheral phenylalanine levels, DASS depression scale scores, or DASS anxiety scale scores, implying that salivary serotonin does not reflect central serotonin turnover. Additionally, this study suggests that salivary serotonin is not a suitable marker for monitoring dietary control, mood, or anxiety in PKU.

Synopsis: Salivary serotonin does not correlate with peripheral phenylalanine levels, DASS depression scale scores, or DASS anxiety scale scores, suggesting that salivary serotonin is not a suitable marker for monitoring dietary control, mood, or anxiety in PKU.

1. Introduction

Since the discovery of Phenylketonuria (PKU) in 1934 by Ivar Asbjørn Følling, much research has been invested in the management of this inborn error of metabolism. While the mainstay of treatment is centered on protein-restricted diets, other therapies have been researched and utilized, such as BH_4 supplementation, large neutral amino acids (LNAA), and glycomacropeptides (GMP). Recent developments include gene therapy and enzyme substitution or replacement [1].

Continued research is necessary because of suboptimal cognitive and executive function, as well as increased risk of behavioural and mood disorders in those with poor dietary management. Even in early-treated children and adolescents, psychological and psychiatric complications such as depression, anxiety, stress experience, and reduced self-esteem along with attentional impairment, restricted scholastic performance and achievement, executive functioning limitations, and concomitant restricted autonomy are commonplace. In adulthood, conditions such as generalized anxiety, phobias, depressed mood, social maturity deficits, and social isolation are common barriers to functioning and quality of life [13,40].

From a biological standpoint, elevations in phenylalanine (Phe) levels probably do not directly cause the psychiatric symptoms. There are three likely mechanisms through which hyperphenylalaninemia affects

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brain function indirectly:

1. Elevated Phe concentrations have a neurotoxic effect that in turn contributes to structural brain damage and inhibition of myelin development in poorly-treated children and dysmyelination in adults who discontinue the Phe-restricted diet [36]. Even children with early-treated PKU can have white matter changes on MRI [3]. Neuroimaging studies demonstrate high-signal intensity in the periventricular white matter, which can extend into subcortical and frontal regions in more severe cases. The histopathology of these lesions reveals that diffuse white matter pathology in untreated patients reflects hypomyelination, while in the early-treated patients is more likely due to intramyelinic edema, or intracellular accumulation of a hydrophilic metabolite [23]. This pathology is associated with metabolic control and may be reversed if patients adhere to a strict Phe-restricted diet [4].
2. Increased concentrations of Phe overwhelm the common amino acid transport system into the brain, which cause the failure of import of several amino acids, such as tyrosine and tryptophan. [22]. It is well known that large neutral amino acids (LNAA), including phenylalanine, compete for transport across the blood-brain-barrier via the L-type amino acid carrier. The direct effects of the elevated brain Phe and depleted LNAA are probably the cause of the abnormalities in brain development and function seen in untreated PKU. When the plasma LNAAs are increased, the influx of Phe into the brain may be lowered [37].
3. Reductions of brain tyrosine and tryptophan lead to reductions in brain dopamine and serotonin, since the former are precursors of the latter. In turn, this neurotransmitter deficiency is associated with depression (serotonin deficiency) and behavioural disturbances (especially frontal lobe dysfunction in dopamine deficiency) [35]. Sleep disturbances in PKU patients have been also well documented [11]. Bioavailability of tyrosine due to changes in intestinal microbiota and altered metabolism of tryptophan via the kynurenine pathway in patients who are ingesting medical foods also contribute to the bioamine defects [32]. Finally, animal studies have been extremely useful to investigate and demonstrate the mechanisms by which elevated Phe affects the brain, discussing the importance of changes in protein synthesis, transport of LNAA, synthesis of monoamine neurotransmitters and activity of glutamate receptors amongst other mechanisms [28].

Currently, there is no reliable biochemical marker that can be used as surrogate for chronic symptoms of depression and anxiety in patients with PKU. Rather, we use self-report assessment tools for psychiatric conditions to identify these symptoms. A few previous studies assessed the utility of CSF dopamine and serotonin in PKU patients [6,20], but the invasiveness of this test renders it unsuitable for routine monitoring. Thus, we sought to determine the relationship between salivary serotonin levels and psychological symptoms associated with depression and anxiety in young adults with PKU. Our hypothesis was that salivary serotonin would correlate with these psychological entities and could be used as a non-invasive marker of central serotonin activity in individuals with PKU. Positive correlation between platelet and CSF serotonin has been demonstrated before in non-PKU patients and rats, but no studies looking for correlation between salivary and CSF serotonin levels were done [5].

2. Methods

2.1. Ethics and recruitment

Institutional research ethics board (UBC Ethics Board, ID# H12–01687) approval was obtained, after which we recruited and obtained informed consent from 20 adults with early-treated PKU who had been treated in our Adult Metabolic Diseases Clinic. Exclusion criteria included individuals on Selective Serotonin Reuptake Inhibitors (SSRIs), considering the effect of these drugs of lowering peripheral serotonin levels [2].

2.2. Phe level and salivary sampling

At a regular follow-up clinic visit, a blood spot Phe level was drawn and a clean pipette sample of approximately 0.2–0.5 mL of saliva was drawn from the mouth the morning of that clinic visit, between 8 and 11 AM, which is the peak salivary serotonin secretion [39]. Phenylalanine analysis was performed on a Biochrome amino acid analyzer (with ion exchange column). Patients were instructed to avoid heavy exercise, sexual intercourse, alcohol, caffeine, and cheese on the sampling day. They were also required to brush their teeth without toothpaste and to rinse their mouth with water 10 min before sampling. We have also ran 3 saliva samples as control from 3 non-PKU adults.

2.3. Average Phe, Average Tyr, and BMI

Average Phe levels over the previous two years were calculated for each individual to ensure representative Phe levels. Similarly, average Tyrosine (Tyr) levels for the previous two years was calculated to exclude nutritional tyrosine deficiency. Height and weight were used to calculated body mass index (BMI).

2.4. DASS: Depression, Anxiety and Stress Scale

Study participants completed a short psychometric instrument that evaluates psychological symptoms commonly experienced by PKU populations. The DASS is a validated 42-item self-report instrument developed as a clinical and research tool to measure core symptoms as conventionally defined within three negative clinically significant emotional states (typically described as depression, anxiety, and stress) [10,16,25]. Respondents were asked to rate each item according to a 4-point scale with regard to the extent over the previous week that each statement applied to them. Subscale scores for each of the three symptom constellations were calculated by summation. Scores between 0 and 9 for depression and between 0 and 7 for anxiety are considered to be normal, whereas scores above the respective cutoffs are categorized in the scale manual as mild, moderate, severe or extremely severe.

2.5. Salivary serotonin and cortisol

Salivary serotonin levels were determined by Serotonin Ultrasensitive ELISA using the Eagle Biosciences Kit (Catalog#: SEU39-K01, lot SES 109). Three replicates were conducted for each sample. Salivary cortisol levels were determined by Salivary Cortisol ELISA using the Eagle Biosciences Kit (Catalog#: OR32-K01, lot 3809). One replicate was conducted for each sample. The Eagle Biosciences Program (elisaanalysis.com) was used to plot photometric data and extrapolate salivary serotonin and cortisol levels.

Statistical analysis involved calculation of the means and standard deviations for the measured parameters. SAS JMP 13.1.0 was used for all statistical analyses.

Normality of the data was determined using the Shapiro-Wilks test. Student's *t*-test for normally distributed data (plasma Phe levels and age) and Wilcoxon's rank sum test for non-normally distributed data (the remaining 8 variables) were used to determine if there were statistically significant differences between males and females. Non-parametric Spearman's ρ correlation analysis was applied given the majority of the data were not normally distributed between the 10 continuous variables (age, plasma Phe level, DASS depression score, DASS anxiety score, BMI, salivary serotonin, salivary cortisol, 2-year average Phe, 2-year average Tyr, 2-year average Phe:Tyr ratio).