

The Use of Prealbumin Concentration as a Biomarker of Nutritional Status in Treated Phenylketonuric Patients

Júlio César Rocha^a Manuela Ferreira Almeida^a Carla Carmona^a
Maria Luís Cardoso^a Nuno Borges^b Isabel Soares^c Graça Salcedo^c
Margarida Reis Lima^a Isabel Azevedo^d Francjan J. van Spronsen^e

^aCentro de Genética Médica Jacinto de Magalhães, INSA, ^bFaculdade de Ciências da Nutrição e Alimentação da Universidade do Porto, ^cLaboratório Endoclab, ^dDepartamento de Bioquímica, Faculdade de Medicina da Universidade do Porto, Porto, Portugal; ^eBeatrix Children's Hospital, University Medical Centre of Groningen, University of Groningen, Groningen, The Netherlands

Key Words

Phenylketonuria · Prealbumin · Amino acid mixture · Natural protein · Nutritional status

Abstract

Background/Aims: The neurological sequelae resulting from untreated phenylketonuria are diminished by the success of early introduced and continued dietary treatment. Nowadays, nutritional status is gaining importance in the follow-up of these patients. The aim of this work was to study the relevance of prealbumin concentration as biomarker of protein nutritional status of phenylketonuric patients. **Methods:** We collected data from 69 phenylketonuric patients on food intake, blood prealbumin and blood phenylalanine concentrations. Protein insufficiency was defined as prealbumin z-scores below the 5th percentile of reference population. Additionally, we considered a prealbumin concentration of 20 mg/dl as a threshold level. **Results:** Nine patients (13%) showed signs of protein insufficiency. When the threshold of 20 mg/dl for prealbumin was used, we found 38 patients (55%) with low prealbumin concentrations. **Conclusion:** A significant group presented signs of protein insufficiency either using prealbumin z-scores or prealbumin con-

centration threshold, especially in milder forms of the disease. The results of this seem to confirm the already described threshold level for prealbumin concentration, suggesting that its measurement may be important for nutritional status evaluation, preventing protein insufficiency in milder forms of phenylketonuria.

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Introduction

Phenylketonuria (PKU; MIM No. 261600) is the most frequent inborn error of amino acid metabolism with a prevalence of 1:10,914 in Portugal [1]. The initial aim of the treatment was prevention of mental retardation, which is well achieved [2]. Nowadays, other topics of concern have emerged in the follow-up of these patients. Among these, the impact of the severe restriction of normal, natural protein-containing food on the nutritional status is one of the most important [3–6]. The more restrictive the diet in natural protein, the more the patients depend on the phenylalanine-free amino acid mixture (AAM) to satisfy their nitrogen needs. However, the AAM has a rather poor efficacy of utilization compared

Table 1. Nutritional recommendations for PKU patients

Daily needs ¹		<6 months	6–12 months	1–4 years	4–7 years	7–11 years	11–15 years	15–19 years
Energy	kcal/kg	145–95	135–80	–	–	–	–	–
	kcal	–	–	1,300	1,700	2,400	2,200–2,700	1,800–2,100
Total protein	g/kg	3.0–3.5	2.5–3.0					
	g			30	35	40	50–55	50–65
Carbohydrate	g	30–35% of daily energy		50–60% of daily energy				
Lipids	g	50% of daily energy		35% of daily energy				
Phenylalanine	mg/kg	20–70	15–50	15–40	15–35	15–30	15–30	10–30

¹ Adapted from Elsas and Acosta [13].

with intact or natural protein-containing formulas [7]. Therefore, PKU patients are at risk of developing nutritional deficiencies, especially of protein and protein-related nutrients. Consequently, it is important to find a parameter that is a useful indicator of the protein status.

In a population of individuals at risk of nutritional deficiencies, prealbumin seems to be a good indicator of protein status because of its short half-life of 2 days [8]. Low levels as observed in protein-energy-malnutrition syndromes are explained by low synthesis rate [8, 9]. In fact, children with restricted intake of protein and energy usually develop an adaptive response in order to decrease the rate of synthesis [8, 10].

Low prealbumin concentrations were previously reported in PKU patients [5, 6, 11]. Arnold et al. [6] demonstrated that lower prealbumin concentrations were found in the youngest PKU patients, possibly as a result of the higher needs for growth and development. Strict diet and better compliance, however, are also associated with lower concentrations of prealbumin [5].

The aim of this study was to further investigate prealbumin concentrations in PKU patients, and its possible relationship with protein intake and metabolic control.

Materials and Methods

The Centro de Genética Médica Jacinto de Magalhães is responsible for the Portuguese neonatal screening and is one of the national treatment centres of PKU. At this centre, PKU patients' management protocol comprises an analytical evaluation once a year which includes several hematological and biochemical parameters. On the same day, patients are observed by a physician, nutritionist and psychologist.

From all the 145 patients in the follow-up, one of the authors (J.C.R.) studied retrospectively the records of a group of 69 treated PKU patients in whom prealbumin concentrations were mea-

sured within the same reference laboratory (Endoclab, Porto, Portugal). The remaining 76 patients were not included because data were either not available or measured elsewhere. Patients' conditions were according to the Declaration of Helsinki. (<http://www.wma.net/e/policy/pdf/17c.pdf>). Approval of the medical ethical committee was not considered necessary as the retrospective studies and laboratory investigations were part of routine care, and data were used anonymously.

Data on prescribed amount of AAM were collected from the records of the last appointment prior to the prealbumin measurement. The prescribed amount of AAM was defined based on the nutritional recommendations of the Medical Research Council Working Party on PKU [12], adopted by the Portuguese guidelines [1], and on the patient's tolerance of phenylalanine. The AAM prescribed in all patients were PKU2[®] and PKU3[®] (Milupa, Friedrichsdorf, Germany). PKU2 was prescribed in patients aged between 1 and 12 years, while PKU3 was prescribed from 12 years onwards. Carbohydrate, lipids and energy intake adjustments were based on nutritional recommendations presented in table 1 [13]. Energy intake was adjusted, taking into account the anthropometric, body composition and analytical evaluations.

Metabolic control of the patients was defined as the median of phenylalanine blood concentrations in the last year preceding the measurements of prealbumin. The number of control blood samples depended on age, frequency of necessity of dietary changes and compliance of the individual patient. Blood phenylalanine levels were measured in blood spots collected at home as well as in venous blood samples collected at outpatient clinic visit. All phenylalanine concentrations were measured from blood spots using the phenylalanine dehydrogenase-coupled enzymatic assay (Quantase[®], Hemel Hempstead, UK) [14, 15], with an error less than 8.7% in our laboratory.

Analytical data for prealbumin concentrations were collected at regular yearly clinical evaluation. Prealbumin concentrations were measured within the same reference laboratory (Endoclab, Porto, Portugal) using the same method for all patients. Prealbumin concentrations were determined by nephelometry; calibration: IFCC Certified Reference Material 470 (CRM 470) with total biological error of 7.92%. The prealbumin concentrations were transferred to z-scores using reference data [16]. Prealbumin z-scores below the 5th percentile were classified as protein in-

sufficiency. Additionally, we used a prealbumin concentration threshold level of 20 mg/dl already described [5, 17].

Statistical analyses were performed using SPSS v11.5.0. We used descriptive and exploratory analyses and the Kolmogorov-Smirnov test in order to verify the normal distribution of the variables. To find the association between continuous variables, Pearson's correlation was used. Results were considered significant when $p < 0.05$.

Results

The studied group of 69 patients consisted of 30 females (43.5%) and 39 males (56.5%) aged 1–27 (mean = 10; SD = 6.47) years of age. The age at diagnosis varied between the 3rd and the 11th day of life (mean = 6; SD = 2). The diagnostic blood phenylalanine concentration varied between 3.3 and 60 mg/dl (1 mg/dl = 60 μ M; mean = 14.1; SD = 9.7). Patients were classified according to the neonatal screening concentration of blood phenylalanine as follows: hyperphenylalaninemia (HPA; between 3 and 6 mg/dl; $n = 14$), mild PKU (between 6 and 20 mg/dl; $n = 32$) and classical PKU (equal or above 20 mg/dl; $n = 19$), according to the Neonatal Screening Committee in Portugal and the Portuguese Society of Metabolic Disorders [1]. Four patients were diagnosed later because they were born before the start of neonatal screening in 1979.

The mean (SD) of the prealbumin z-score was -0.5248 (1.09), which is significantly below z-score = 0 ($p < 0.001$). Nine patients (13%) had a prealbumin z-score below the 5th percentile (-1.64), and one above the 95th percentile ($+1.64$; fig. 1). Of these 9, one had classical PKU, 5 mild PKU and 3 HPA. All patients with z-score of prealbumin below the 5th percentile were younger than 15 years of age. When the classification of PKU was taken into account, 5% of patients with classical PKU, 16% with mild PKU, and 21% with HPA showed prealbumin z-scores below the 5th percentile without statistically significant differences within these three groups ($p = 0.237$).

Until 2006, in 5 of the 14 patients with HPA it was decided not to prescribe AAM. Two of these 5 had low prealbumin concentrations. In the more recently born 9 patients with HPA, at least a small amount of AAM was prescribed. Of these 9, one had low prealbumin concentrations. All the other 64 patients (93%) had an AAM prescribed. We did not find a statistically significant correlation between prealbumin z-score and the amount of AAM (PKU2 or PKU3) prescribed (between 0.48 and 2.79 g of amino acid \cdot kg $^{-1}$ \cdot day $^{-1}$; $R^2 = 0.01$; $p = 0.38$).

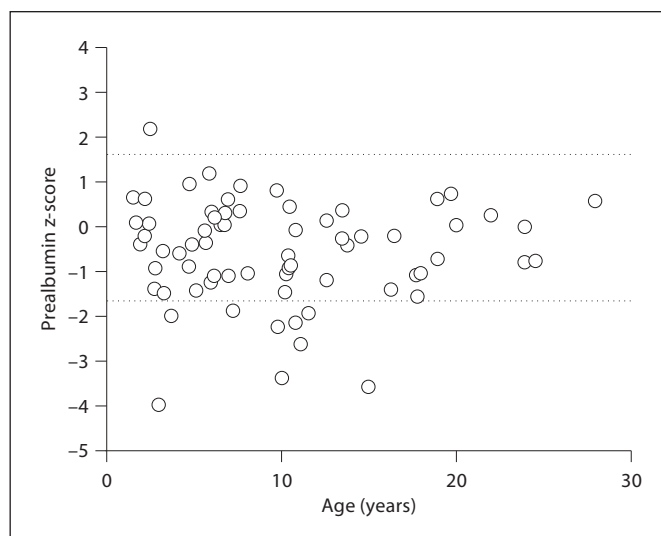


Fig. 1. Prealbumin z-scores of patients. Horizontal lines correspond to the 5th (z-score = -1.64) and 95th (z-score = $+1.64$) percentiles of prealbumin.

Prealbumin z-score and the median of the individual blood phenylalanine concentrations (metabolic control) were not statistically significantly correlated ($R^2 = 0.003$; $p = 0.65$).

When we used a prealbumin concentration threshold of 20 mg/dl, we found 38 patients (55%) with low prealbumin. The χ^2 test showed an association between prealbumin below 20 mg/dl and age below 15 years ($p < 0.0001$). Prealbumin z-score averages were not different in these two age groups ($p = 0.488$). Prealbumin concentrations were significantly higher in the group of older patients compared to the group of patients younger than 15 years (25.53 ± 4.38 vs. 18.38 ± 4.12 ; $p < 0.001$). This difference is in good agreement with the reference data for the normal population [16].

Discussion

This study identified 9 patients out of a group of 69 (13%) with prealbumin z-scores below the 5th percentile, i.e. with protein insufficiency. Mean prealbumin z-score was significantly below zero, i.e. negative. Z-scores of prealbumin concentrations were not statistically correlated with amount of amino acid intake, metabolic control of the patients, or with clinical severity of PKU. With respect to age, it is interesting that all the patients with protein insufficiency were younger than 15 years. When us-

ing the threshold level, we found 38 patients (55%) with prealbumin concentration below 20 mg/dl.

Before discussing the results in more detail, some methodological issues need to be addressed. First, the definition of protein insufficiency in our study (prealbumin z-score below the 5th percentile) was different from the definitions used by Arnold et al. [5] (prealbumin concentration below 20 mg/dl), or the values of marginal (15–20 mg/dl) or deficient (<15 mg/dl) intake used by Acosta [4]. In contrast, we used the data from a large cohort as reference values [16], which gives us the distribution by age and gender of specific ranges for normal prealbumin. However, we also considered the prealbumin threshold level of 20 mg/dl already described in the literature [5]. When the cutoff value of 20 mg/dl was used, 38 (55%) of our patients would have been classified as having low prealbumin. Second, we used the terminology ‘protein insufficiency’, as used by Arnold et al. [5, 6], considering that ‘protein deficiency’ should be adopted only in the case of clinical symptoms. Third, the AAM intake in this study reflects the prescribed rather than the actual intake. Especially in older patients, the prescribed intake and actual intake may differ. Therefore, these data on the relationship between intake and prealbumin z-score should be considered with caution.

Our results on the frequency of protein insufficiency as defined by Arnold et al. [5] are comparable (55% in our study vs. 42% in their study). This is remarkable because the sample of Arnold et al. [5] was composed only by patients with the classical form of the disease; our results of classic PKU did not show such high prevalence of protein insufficiency. Nevertheless, that comparison used prealbumin concentration instead of prealbumin z-score. From a theoretical point of view, we would prefer using z-score of prealbumin instead of using their absolute concentration because of the known variation with sex and age.

Our data show that all the patients with protein insufficiency (13%) were younger than 15 years (fig. 1), suggesting the large importance of sufficient protein intake during periods of high growth velocity. Additionally, it seems very important to mention that the mean prealbumin z-score was significantly negative, suggesting a global tendency for protein insufficiency. The previously described threshold level for prealbumin, which was confirmed again in this study, may be important to consider in clinical practice in order to prevent the development of protein insufficiency. Considering the results of the study of Arnold et al. [5], it would be advisable to maintain prealbumin concentrations above 20 mg/dl in patients with PKU in order to guarantee a good protein status for growth.

In day to day care by PKU teams, attention is easily focused on patients with the most severe form of PKU. As already shown above, 8 of the 9 patients identified with protein insufficiency had mild PKU or HPA. In the group of HPA patients, especially those without AAM, protein insufficiency was observed. Therefore, the present results indicate that in the milder phenotypes it is also important to evaluate protein status and prescribe sufficient amounts of AAM because the foods chosen are usually low in high biologic value protein, like fruits and vegetables. In other words, natural protein restriction may be enough to guarantee an acceptable metabolic control, but clearly insufficient to maintain a good nutritional status. On the other hand, the relatively lower prevalence of protein insufficiency in the classical PKU may be justified by the increased amounts of prescribed AAM (between 1 and 2 g of amino acid per kg and per day), spread out over several times a day and together with some natural protein, according to the nutritional recommendations [1, 12].

Prealbumin is a reliable indicator of nutritional protein status in general and of the adequacy of the AAM intake due to its short biological half-life (e.g. 2 days vs. 20 days for albumin). Prealbumin insufficiency is also related to decreased plasma zinc concentrations, increased hepatic enzymes (AST and ALT), inflammatory processes, and trauma [8]. No sign of any of these alterations was observed in our population (results not shown). Hence, the lower prealbumin status might be related to some tryptophan deficiency, which could be relevant to the still not completely normal neuropsychological findings in PKU patients [18].

Conclusion

As the prevention of neurological sequelae of PKU patients can be achieved with neonatal screening programs and continuous treatment, health improvement of this population should include optimization of the nutritional status. We have demonstrated that at least some patients manifest protein insufficiency, as shown by their prealbumin concentrations, regardless of disease severity. Therefore, measurement of plasma prealbumin may identify protein insufficiency. Further studies are necessary to investigate the possibility of improving the quality of treatment to optimize protein nutritional status in patients with all degrees of PKU severity.

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