

CHANGES IN THE COMPOSITION OF SALIVARY AMINOACIDS, PROTEINS AND ENZYMES IN CHILDREN WITH PHENYLKETONURIA

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Summary

Since children with phenylketonuria have a low incidence of caries, the aim of this study was to examine the changes occurring in the aminoacid and protein composition as well as in the composition of salivary enzymes, which might serve as biochemical indicators, representing the factors of predisposition to the development of caries.

For this purpose, the analysis was carried out in the native, unstimulated, mixed salivary specimens taken from the sublingual region of children with late diagnosis of PKU ($n = 16$), those with early diagnosis of the disorder by means of a systematic diagnostic screening program for PKU using Guthrie's test ($n = 7$), and a control group of children free of any disturbances of the aminoacid metabolism ($n = 44$).

A method of qualitative chromatography on silica gel was used for separation of aminoacids, while the proportions of each individual aminoacid were determined by a densitometer. The content of total salivary proteins was assessed by the method of Lowry, and the concentration of lysozyme by the method of Prockop.

Differences in particular proportions of aminoacids, and an increase in the total protein value as well as in the lysozyme activity, could be taken as the factors of predisposition or resistance to caries.

Key words: phenylketonuria, saliva, caries

INTRODUCTION

Phenylketonuria (PKU) is an aminoacid metabolism disturbance in which phenylalanine cannot be converted to tyrosine due to a defect in phenylalanine hydroxylase, an enzyme exclusively found in the liver. If untreated, PKU leads to severe mental retardation (IQ usually below 50) in about 99% of patients.

Today, this disturbance in the phenylalanine metabolism can be detected early during infancy, i. e. as early as the first week of the newborn's life, using Guthrie's test. Thus, applying systematic neonatal screening programs for PKU, children with this disorder can be timely detected and allowed to grow and develop normally, providing they have been put on an appropriate diet (i. e. food with a very low content of fatty acids (FA)) (1—4).

Previous studies on the protein and aminoacid composition of saliva have pointed to a constant correlation existing between the aminoacid composition of saliva and the incidence of caries (5). Some of the studies on the incidence of caries in children with late diagnosis of PKU have revealed them to have much less or practically no caries as compared to normal children. Since caries is still one of the most significant problem encountered in the pedodontic pathology, a study of the causes and progression of caries appeared to be of an utmost importance, the more so as the basic causes of changes in mineralization and incipient demineralization of hard dental structures have not yet been elucidated.

Besides general factors, the local factors of tooth environment are also important. Therefore, saliva as a medium surrounding the structures within the mouth is an optimal material for the analysis of both local and some general biochemical indicators such as aminoacids, proteins and enzymes.

THE AIM OF THE STUDY

The aim of this study was to examine the changes occurring in the aminoacid and protein composition, as well as in the composition of salivary enzymes which might serve as biochemical indicators, representing the factors of predisposition to caries. For this purpose, the analysis was carried out in the native, unstimulated, mixed salivary specimens of children with late diagnosis of PKU (n — 16), those with early diagnosis of the disorder, made by means of a systematic screening program for PKU (n — 7), and a control group of normal children, i. e. those without any disturbance of the aminoacid metabolism (n — 44).

MATERIAL AND METHODS

Saliva specimens were taken using a sterilized syringe, put into cooled phials and immediately frozen until analysis.

A method of qualitative chromatography on silica gel was employed for separation of aminoacids, while the proportions of individual aminoacids were determined by a densitometer. The content of total salivary proteins was determined by the method of Lowry (6), and the concentration of lysozyme by the method of Prockop (7).

RESULTS AND DISCUSSION

The following aminoacids were detected in the unstimulated saliva specimens of all the three groups of children: histidine, lysine, glutamine,

glycine, serine, glutamic acid, threonine, alanine, valine, phenylalanine and leucine (Fig. 1).

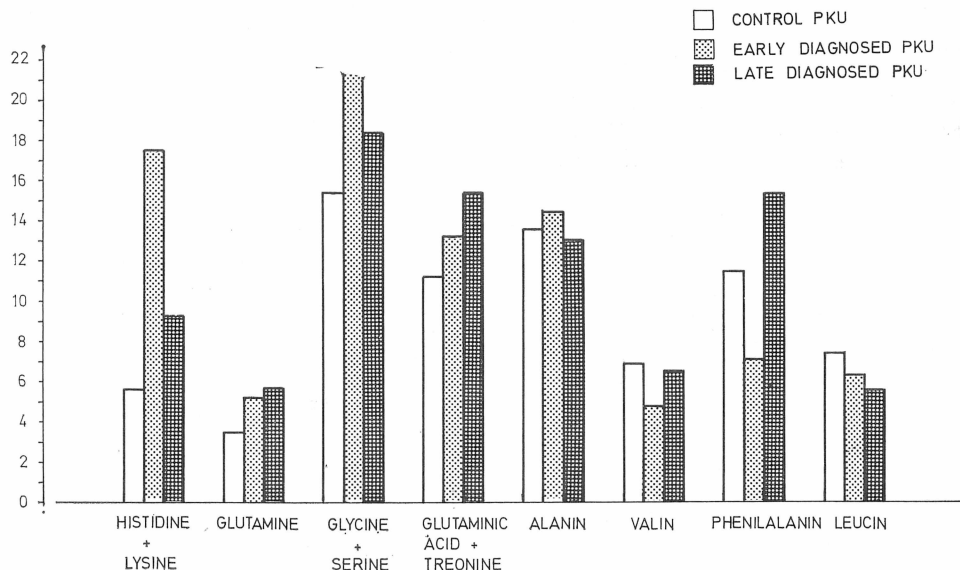


Figure 1: Proportions of Aminoacids in Children's Saliva

The results obtained by qualitative chromatography indicated higher proportions of histidine, lysine, glutamine, glycine, serine, glutamic acid, threonine and, especially, phenylalanine in children with PKU (both early and late diagnosis of PKU), whereas the proportions of valine and leucine were found to be lower.

In the saliva of children with early diagnosis of PKU, higher proportions of histidine, lysine, glycine, serine and alanine were observed as compared to those recorded in the saliva of either children with late diagnosis of PKU or of normal children.

The total protein values were higher in children with PKU, particularly in those with untreated PKU, in whom these values were found to be twofold those recorded in normal children (Fig. 2).

The lysozyme enzyme activity is particularly important and it was found to be twice as high in the saliva of children with either early or late diagnosis of PKU as in the saliva taken from normal children (Fig. 3).

Lysozyme is a mucolytic enzyme with a small molecular weight. It is a cationic protein which can be found in secretions and cells of the body, especially in tears. This enzyme has the ability of depolymerization of mucopolysaccharides on cellular walls of bacteria. It is supposed to be released from lysozymes of phagocytes actively secreted by monocytes and macrophages.

Therefore, a higher activity of this enzyme probably plays an important role of a factor of resistance to caries in the body of children with PKU who are practically free of caries, and appears to actually represent the front line of the body defense against the disease. This, however, requires further studies.

According to the literature data available, there are two possible explanations for this phenomenon of resistance to caries in children with PKU. One of them was proposed by Ogresta and Zaninović. (8) As Guthrie's test is based on the inhibition of growth of *Bacillus subtilis* under the in-

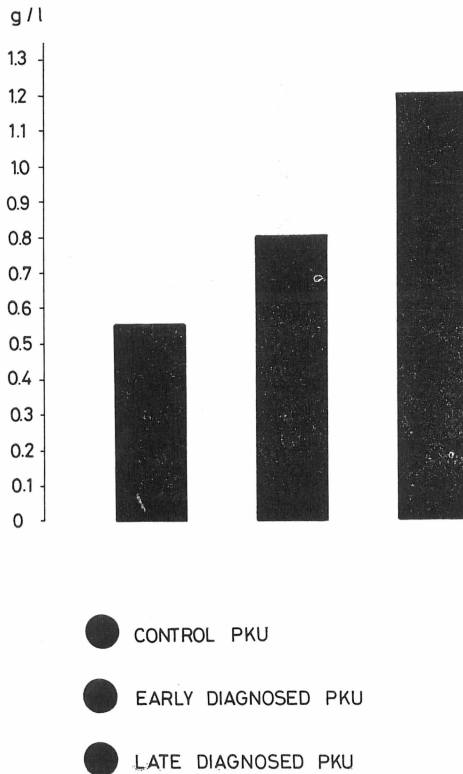


Figure 2: Total Proteins (by Lowry)

fluence of B-2 phenylalanine and the antagonistic effect of phenylalanine, increased phenylalanine could also be supposed to inhibit the growth of other cariogenic microorganisms (8).

The other hypothesis, based on Bickel's findings, would be that lower values of phenylalanine might result in a reduced maturation of bone. Thus, an increased quantity of phenylalanine could be supposed to condition good mineralization of hard dental structures, which would, in turn, increase resistance to caries.

mg/l hum. lys.

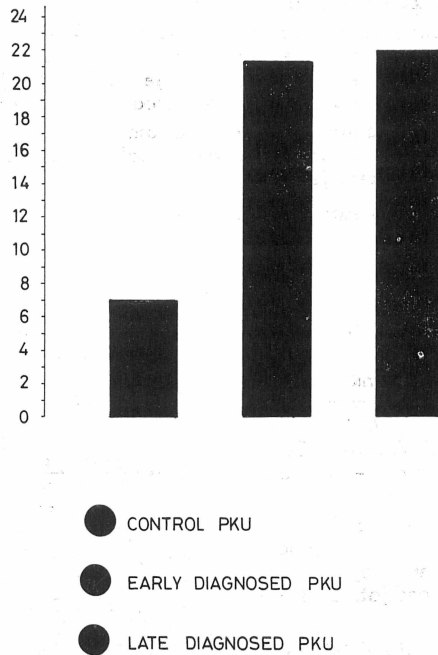


Figure 3: Lysozyme (by Prockop)

Since bones and teeth consist of the same inorganic substances differing in their percentages only, deposition of substances in both the bone and the tooth is identical. Therefore, it could be supposed that phenylalanine, along with proteins, also plays an important role in ossification and mineralization.

In accordance with all said above, an increased activity of lysozyme, differences in particular proportions of aminoacids, and an increase in the total protein value could be taken as the factors of predisposition or resistance to caries.

PROMJENE U AMINOKISELINAMA, PROTEINIMA I ENZIMIMA U DJECIMA S FENILKETONURIJOM

Sažetak

Budući da djeca oboljela od fenilketonurije imaju malu incidenciju karijesa, željeli smo ispitati promjene u sastavu aminokiselina, proteina i fermenta, koje bi mogle — kao biokemijski indikatori — biti predispozicijski činioci za razvoj karijesa. U tu svrhu analizirana je nativna slina sublingvalnog

područja u uzrocima djece s kasno otkrivenom fenilketonurijom (n — 16), djece s rano otkrivenom bolešću u sistematskom traganju za fenilketourijom primjenom Guthrie testa (n — 7) te djece kontrolne skupine bez poremećaja u metabolizmu aminokiselina.

Aminokiseline su razdvajane metodom tankoslojne kromatografije na silika gelu, a proporcije pojedinih aminokiselina određene su denzitometrijski. Sadržaj ukupnih proteina sline određeni je po Lowryevoj metodi u mg/dl sline. Za određivanje lizozima korištena je metoda po Prockopu.

Razlike u pojedinim proporcijama aminokiselina, kao i povišeni ukupni proteini i povećana aktivnost lizozima, mogle bi se označiti kao faktor sklonosti, odnosno rezistencije prema karijesu.

Ključne riječi: fenilketonurija, slina, karijes

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