Medical Nutrition Therapy and Intestinal Microbiota in Phenylketonuria

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Abstract

The healthy intestinal microbiota has crucial effects, such as protection from pathogenic factors and the development of the immune system. The composition, diversity, and functioning of the microorganisms that make up the microbiota, change at short notice with dietary factors. In this respect, medical nutritional therapies applied in congenital metabolic diseases play a crucial role in shaping the intestinal microbiota. These dietary interventions, with their unique macronutrient and micronutrient compositions, interact with the microbiota, modulate immune responses, and alter the protective integrity of the gut epithelial barrier. In phenylketonuria, the most common amino acid metabolism disorder, it is thought that there may be differences in the microbiota due to the phenylalanine-restricted diet therapy that must be applied throughout life, and studies have been carried out on this subject. Studies have concluded that differences in microbial diversity exist in phenylketonuria patients, although it is not yet known whether it is a result of the disease itself or dietary therapy. This review is intended to examine the medical nutritional therapy administered in phenylketonuria and its effects on the intestinal microbiota.

Keywords: Intestinal microbiota, nutrition therapy, phenylketonuria.

Fenilketonüride Tıbbi Beslenme Tedavisi ve İntestinal Mikrobiyota

Öz

Sağlıklı intestinal mikrobiyota, hastalık yapıcı etkenlerden korunma ve bağışıklık sisteminin gelişimi gibi birçok faydalı etkiye sahiptir. Mikrobiyotayı oluşturan mikroorganizmaların bileşimi, çeşitliliği ve işleyişi, diyet faktörü ile kısa sürede değişmektedir. Bu doğrultuda doğumsal metabolizma hastalıklarında uygulanan tıbbi beslenme tedavileri intestinal mikrobiyota için önemli bir etmendir. Bu diyet müdahaleleri sahip olduğu içerik nedeniyle mikrobiyota ile etkileşime girmekte, bağışıklık yanıtını etkilemekte ve bağırsak bariyerinin koruyucu fonksiyonlarında değişikliğe yol açmaktadır. Aminoasit metabolizma bozuklukları içerisinde en sık görülen fenilketonüride, yaşam boyu uygulanması gereken fenilalaninden kısıtlı diyet tedavisi nedeniyle mikrobiyota açısından farklılıklar oluşabileceği düşünülmüş ve bu konuda çalışmalar yapılmıştır. Çalışmalar sonucunda hastalığın kendisinin mi yoksa diyet tedavisinin mi bir sonucu olduğu henüz bilinmese de, fenilketonüri hastalarında mikrobiyal çeşitlilikte azalma ve farklılıklar olduğu sonucuna varılmıştır. Bu derlemede, fenilketonüride uygulanan tıbbi beslenme tedavisinin ve intestinal mikrobiyota üzerine etkilerinin incelenmesi amaçlanmıştır.

Anahtar Sözcükler: İntestinal mikrobiyota, beslenme tedavisi, fenilketonüri.

Introduction

Phenylketonuria (PKU) is an inherited metabolic disease caused by defects in the phenylalanine hydroxylase (PAH) enzyme. In these patients, in addition to clinical findings such as light hair, eyes, and skin color, irreversible mental retardation occurs if an early diagnosis is not made and therapy is not started. Early diagnosis and therapy

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are very important to prevent this condition. Although there are many therapy methods, the gold standard for phenylketonuria is phenylalanine-restricted diet therapy. These patients are treated with a nutritional therapy that includes special medical formulas limited in phenylalanine and rich in tyrosine, and special low-protein products. The fact that individuals with PKU receive disease-specific medical nutritional therapy and that their diets differ in macro and micronutrient elements quickly results in changes in the microbiota[1].

The healthy intestinal microbiota is in a state of balance in terms of microorganisms. Factors such as mode of delivery, genetic predisposition, environmental exposure, use of probiotics, prebiotics, and antibiotics, as well as nutrition and diet, significantly influence the intestinal microbiota[2]. This is why in PKU, one of the congenital metabolic diseases, conditions such as whether an individual received breast milk as an infant, and the medical formulas they used, the complete removal or limitation of certain nutrients in the diet constitutes diversity and differences in the microbiota[3]. This study aims to examine the effects of medical nutritional therapy administered in phenylketonuria on the intestinal microbiota.

Phenylketonuria

Phenylketonuria is an autosomal recessive metabolic disorder caused by a deficiency in tetrahydrobiopterin (BH_4), the cofactor of the phenylalanine hydroxylase (PAH) enzyme, or the PAH enzyme itself, which is essential for converting phenylalanine to tyrosine[4]. PKU, the most common disorder of amino acid metabolism, was discovered in the 1930s when Asbjørn Følling identified phenylpyruvic acid in the urine of two children with mental retardation during tests for ketonuria. In 1934, biochemist and physician Asbjørn Følling described PKU disease as an "inherited metabolic disease characterized by severe mental disorder, motor problem, and skin abnormalities" and called it "imbecillitas phenylpyruvica"[5]. The disease was named "Phenylketonuria" by Lionel Penrose in 1935[5]. The first successful nutritional therapy specific to the disease was carried out by Horst Bickel in the 1950s, with a restricted diet from phenylalanine. In the 1960s, Robert Guthrie developed a diagnostic test (Guthrie test) for hyperphenylalaninemia (HPA). This test, which is also being used today, constitutes the neonatal screening test for PKU[6].

Phenylketonuria, which is considered among congenital metabolic diseases, is frequently seen around the world. While the incidence of PKU is 1:10,000 newborns in Europe, the country with the lowest incidence in this region is Finland with 1:100,000 births. Of the Asian countries, the incidence of PKU in China is known as 1:100,500, and in Japan, it is known as 1:70,000 newborns[7]. Due to the high number of consanguineous marriages, this ratio is 1:4,000 in Türkiye and Türkiye has become one of the countries where PKU disease is most common in the world[6].

Phenylketonuria is classified according to the phenylalanine level and enzyme activity at diagnosis. The normal range of blood phenylalanine level is 50-110 µmol/L[1]. It is classified as phenylalanine level is 120-360 µmol/L for Bening HPA, 360-600 µmol/L for Mild HPA, 600-900 µmol/L for Mild PKU, 900-1200 µmol/L for Moderate PKU, >1200 µmol/L for Classic PKU[1]. In addition to these, there are types such as BH_4 Cofactor Defects and Maternal PKU that develop due to defects in the cofactor[8].

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Phenylalanine hydroxylase is an enzyme that enables the conversion of phenylalanine into tyrosine. As a result of defects in this enzyme or cofactor, tyrosine production in individuals decreases and blood phenylalanine levels rise above normal levels. With reduced tyrosine production, melanin synthesis is reduced, and physical findings such as light hair, eyes, and skin color occur in PKU patients[9]. Increased concentrations of phenylalanine in the blood result in increased levels of phenylalanine in the brain and accumulation of phenylalanine in the blood-brain barrier. Consequently, adverse outcomes such as impairment of cognitive function, mental retardation, and neurophysiological disorders are seen in patients[1]. With early diagnosis and therapy of the disease, these problems with brain damage can be prevented[8].

Neonatal screenings are important to ensure early recognition of congenital metabolic diseases. Individuals with PKU do not show symptoms until they consume nutrients containing phenylalanine. The neonatal screening test is therefore carried out with a blood sample taken from the heel in the first 24-48 hours following protein intake[10]. This is confirmed by performing plasma amino acid analysis on individuals with high blood phenylalanine levels in the neonatal screening test. The tandem mass spectrometer (Tandem MS) measures the patient's phenylalanine, phenylalanine/tyrosine ratio, and amino acid profile. If tandem MS is also level of phenylalanine > 120 μmol/L, phenylalanine/tyrosine ratio > 2, a second blood examination should be conducted to clarify whether the patient has a defect in BH_4 synthesis or recycling[11].

Phenylketonuria disease requires an interdisciplinary approach that includes metabolism physicians, pediatricians, nutritionists, and psychologists[8]. The main aim of the therapy is to prevent the deterioration in neurological functions by keeping the blood phenylalanine levels of the patients within the normal range and to ensure the normal growth of the patient. There are many therapy methods such as pharmacological chaperone therapy, large neutral amino acid (LNAA) therapy, glycomacropeptide (GMP), gene therapy, and phenylalanine ammonia-lyase (PAL) enzyme therapy[12]. Along with all these methods, the current therapy for PKU disease constitutes a low-phenylalanine diet that should be maintained for life[13].

Medical Nutrition Therapy in Phenylketonuria

A phenylalanine-restricted diet is considered the gold standard for the therapy of PKU. Principles in nutrition therapy include substitution of low-protein nutrients or phenylalanine-free proteins, prevention of excessive buildup of the phenylalanine in the blood and brain by controlling the phenylalanine on a diet, and development of growth[13]. The PKU diet restricts natural protein-rich foods such as meat, poultry, fish, eggs, cheeses, and legumes, while emphasizing the intake of phenylalanine-free, tyrosine-rich medical formulas, low-protein foods, and specially developed products like certain vegetables, fruits, and fats. Another substance to avoid is food and beverages containing aspartame metabolized into phenylalanine[14].

Breast milk is highly important for these patients, both in terms of growth development and mental development[13]. Studies show that breast milk in individuals with PKU provides significant benefits and that taking it in the first years of life positively impacts mental development in these patients. Consumption of breast milk containing low

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amounts of phenylalanine, along with medical formulas lacking phenylalanine, should be achieved by following blood phenylalanine levels[15].

In this nutritional therapy, where some nutrients are not consumed at all, patients' purchases of energy, protein, and phenylalanine should be followed daily[8]. In the diet, proteins are restricted, and energy is primarily derived from liquid fats and pure carbohydrates such as sugar and starch. In addition, the use of low-phenylalanine and low-protein specially developed products in place of natural proteins is important for energy[13]. Besides medicinal products, naturally occurring GMP protein without phenylalanine can be an alternative for individuals with PKU. A study of GMP-fed rats from whey found decreased levels of phenylalanine in plasma (11%) and in the brain (20%), indicating that GMP could be a good source of protein for individuals with PKU. While studies have mentioned the benefits of GMP in reducing the level of phenylalanine in the brain, providing a prebiotic-like effect, and regulating bone health, more data and further studies are needed on this topic[16].

Nutritional therapy in PKU patients positively affects cognitive and psychological development and enables prevention of mental retardation. Therefore, the restricted diet of phenylalanine is essential for these patients. Meeting patients' protein, energy, vitamin, and mineral requirements, as well as ensuring dietary diversity and diet adherence, is important for individuals with PKU[17]. Studies have shown that patients' age progresses and approaches the adolescent age, along with reduced diet adherence[18]. Patients' aspirations should be considered to ensure compliance with nutritional therapy, the diet should be satisfactory for taste and diversity, dietitians and physicians should give training to the patient and their family on nutritional therapy, amino acid mixtures, special products specific to the disease and change lists and the importance of this therapy should be mentioned[8]. It is also important to monitor the nutritional status and growth development of individuals with PKU by dietitians[19].

Intestinal Microbiota in Phenylketonuria

The microbiota is a community of microorganisms bacteria, fungi, protozoa, archaea, and viruses living alongside humans. After years of association between humans and microorganisms, the human body and gut bacteria formed a symbiotic relationship[20]. The intestinal microbiota, which accounts for 1.5-2 kg of total body weight, contains about 10^{14} microorganisms[21]. This microbiota enables the development of the gut mucosal barrier but has significant effects on human health[22]. A study of rats found that a microorganism-free group was compared with the normal group and that rats in the group lacking microorganisms decreased lymphoid organ development, suppressed immune system, and became more prone to infections[23]. In addition, the intestinal microbiota has a variety of functions, including digestion and absorption of nutrient elements, production of essential amino acids, synthesis, and homeostasis[24]. The regular intestinal microbiota contains 5 phyla, mainly *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, *Verrucomicrobia*, and *Actinobacteria*. The dominant among these bacterial groups are the gram-positive *Firmicutes* phylum and the gram-negative *Bacteroidetes* phylum[25].

Bacteroidetes (*Bacteroides, Prevotella*) is a group of bacteria that enables the formation of short-chain fatty acids such as acetate, butyrate, and propionate by enabling the

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digestion of complex carbohydrates that are resistant to digestive enzymes. *Firmicutes* filum; contains *Clostridium*, *Enterococcus* spp., *Lactobacillus*, *Ruminococcus*, and *Streptococcus* spp. bacteria[26]. While *Streptococcus* spp. and *Enterococcus* spp. are found in low density in the intestinal microbiota of healthy individuals, their density may increase to a level that may cause pathogenic effects in cases of dysbiosis. The *Actinobacteria* phylum which includes *Bifidobacteria* spp. is an essential component of the intestinal microbiota. Since the *Proteobacteria* bacterial group is present in low density in the microbiota of healthy individuals, its increase in number causes dysbiosis and disease risk. This phylum contains the *Enterobacteriaceae* family, *Escherichia coli*, and *Klebsiella* spp. *E. coli* and *Klebsiella* spp. are pathogens that normally occur in low density but increase in number in the case of dysbiosis[27].

The intestinal microbiota of each healthy individual is in a state of balance in terms of beneficial and harmful bacteria. Changes occur in the intestinal microbiota as a result of individual factors such as birth shape, age, genetic status, stress, nutritional style, diet, breast milk, experienced environment, probiotic/prebiotic, and antibiotic use. These factors can cause dysbiosis with degradations such as microorganism content change in the microbiota, and bacterial imbalance[2].

One of the major factors affecting the intestinal microbiota, the feeding style can be divided into subheads such as feeding an individual with breast milk or standard formula, complementary nutrition, and the diet they maintain for life. Breast milk is quite important for the newborn to have a healthy microbiota. Protection against infections plays a role in preventing childhood obesity, the development of the intestinal microbiota, and the mucosal barrier[28]. Although standard formulas developed for infants are developed to have a composition similar to breast milk, studies show that breast-fed infants and standard formula-fed infants have differences in microbiota, while those fed with standard formula have less heterogeneity and diversity[29].

Lifelong dietary practices are the key factor affecting the composition of the intestinal microbiota. The microbiota microorganism community's dietary factor, which causes changes in genus, species, and number, causes differences in the dominant macronutrient elements[28]. In diets high in animal protein and saturated fat, the density of *Bacteroides* increases, and in high-fiber diets, the density of *Prevotella* increases. In diets with high fat and sugar, *Firmicutes* spp. density increased, *Bacteroidetes* spp. the intensity decreases[30,31]. Accordingly, the restriction of natural protein-containing nutrients in the PKU diet, the introduction of pure carbohydrates such as liquid oils and sugar, starch, affect the density of bacteria in the microbiota[14].

The relationship between microbiota, gut health, and diseases was first studied by William Beaumont in 1800 years[32]. Clinical changes in the disease process, as well as medical nutritional therapy where macro or micronutrient items are restricted, affect intestinal microbiota composition due to long-term applications[33]. Recent studies have shown that the composition of intestinal microbiota also changes in PKU patients undergoing restricted medical nutritional therapy from phenylalanine[3,34].

The study by Oliveira et al., compared the intestinal microbiota of normal eating healthy children with PKU who received restricted dietary therapy from phenylalanine and concluded that many groups of bacteria were reduced in the PKU group. In individuals

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with PKU, the dominant groups were *Bacteroidetes* and *Verrumcomicrobia*, while *Firmicutes* filum was found less. Besides this, in the PKU group, there is a decrease in the *Clostridiaceae*, *Erysipelotrichaceae*, and *Lachnospiraceae* families, *Clostridiales* class, *Coprococcus*, *Dorea*, *Lachnospira*, *Odoribacter*, *Ruminococcus,* and *Veillonella* genera; *Prevotella*, *Akkermansia,* and *Peptostreptococcaceae* have increased. *Prevotella* was found to be abundant in the PKU group as well as in healthy children fed a diet low in animal protein and saturated fat[34]. Another study of individuals with adult PKU found that the PKU group had a lower diversity of intestinal microorganisms than the control group. The most dominant filum in both the PKU and control group is *Firmicutes* and *Bacteroidetes*, followed by *Actinobacteria*, *Proteobacteria*, and *Verrucomicrobia*. Also in individuals with PKU, *Lactobacillus*, *Porphyromonas*, *Frisingicoccus*, *Blautia*, and *Faecalibacterium* are among the declining breeds. The study also found that the microbiota of adult PKU individuals differs from that of children[35]. Another study comparing the intestinal microbiota of children with PKU and mild HPA found that *Firmicutes* and *Bacteroidetes* phylums were abundant in both groups, but that *Bacteroidetes* was higher in mild HPA. *Veillonellaceae* was found to have significantly decreased in PKU children, while *Faecalibacterium* and *Ruminococcaceae* were found to have increased in mild HPA. Moreover, an increased *Prevotella/Bacteroides* ratio was observed in mild HPA compared to PKU, which is not statistically important. In contrast, a significant increase in PKU children, *Blautia*, *Clostridium*, and *Lachnospiraceae* were identified. It concluded that *Faecalibacterium* spp., which is significantly increased in the mild HPA group, correlates negatively with fiber intake, both soluble and insoluble fibers[36]. Another study of children with PKU and healthy found that the control group had higher microbial diversity than the PKU group. The most populous breeds in the PKU group were *Bifidobacterium*, *Prevotella*, *Subdoligranulum*, and *Faecalibacterium*, while *Bacteroides*, *Prevotella*, *Faecalibacterium*, and *Bifidobacterium* were the most populous in the control group. Furthermore, *Bacteroides* and *Collinsella* abundance were found to differ significantly between PKU and control groups. The genus *Bacteroidetes* is significantly low in PKU patients, and a strong negative correlation between this genus and blood phenylalanine level was found[37]. Al-Zyoud et al., conducted a study of the presence of *E. coli* in the intestinal microbiota of children with PKU, aiming to compare it against normal intestinal flora. As a result of the analysis concluded that *E. coli* was present in control groups while individuals with PKU were not. There is also a discourse that the absence of *E.coli* in individuals with PKU will cause a shortage of both vitamin B_2 (riboflavin) and vitamin K_2 (menacinone)[38].

A genetically modified probiotic (pHENOMMenal) was administered for therapeutic purposes on PAH^{enu2} mice in a study investigating the function of probiotics in the therapy of PKU. During the first three or four days of probiotic therapy, mice with PKU had reduced blood phenylalanine levels and remained significantly lower compared with untreated controls. As a result, pHENOMMenal may be an inexpensive model for the therapy of PKU[39]. In a pilot study of nine patients (four adults, five children), including five patients with classic PKU and four patients with mild PKU, a microbiota examination was performed on the patients after 6 months of GMP application. As a result of the reviews, GMP has shown a positive effect on beneficial bacteria such as *Agathobacter*, which produces butyrate in the microbiota of patients with PKU. Although GMP is

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presented as a safe alternative that provides a prebiotic-like effect in the PKU diet, studies in larger groups on this topic are needed[40].

Conclusion

The intestinal microbiota, which refers to the microorganisms living in the human intestine, is affected by many endogenous and exogenous factors, especially nutrition. Microbial differences are evident in congenital metabolic diseases that follow a lifelong diet with medical nutritional therapy due to the important role of nutrition on the microbiota. Studies of the most common PKU and intestinal microbiota among amino acid metabolism disorders have shown that microbial diversity is less in individuals with PKU than in healthy individuals, while some bacterial groups have differences. As a result, there are studies indicating that the *Bacteroidetes* phylum is more abundant in individuals with PKU than in healthy individuals and that this phylum has a strong negative correlation with blood phenylalanine levels. In some studies, *Firmicutes* phylum and *Faecalibacterium* have been said to increase in individuals with PKU, while in some studies they have decreased, and *Prevotella* has been reported to increase overall. On the other hand, due to the significant impact of breast milk on the microbiota, it is noted that individuals with PKU may have differences in the microbiota in whether they receive breast milk or not. Given all these studies, there are certainly reductions in microbial diversity in PKU, but it is still unknown whether this is the result of the disease itself or dietary therapy. Further studies investigating the nutritional quality of PKU diets, and their impact on gut microbial ecology and health in greater numbers of patients and for longer periods are needed. Further research on prebiotics, probiotics, and postbiotics in congenital metabolic diseases is needed to create a microbial environment that facilitates disease management and improves quality of life.

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