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Effects of gluten-free, dairy-free diet on childhood nephrotic syndrome and gut microbiota

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Emerging evidence suggests an association between food sensitivity and gut microbiota in children with nephrotic syndrome. Diminished proteinuria resulted from eliminating cow's milk and the use of an oligoantigenic diet which excluded gluten, especially in patients with immune-related conditions, i.e., celiac disease and nephrotic syndrome. The mechanisms underlying the association of diet, gut microbiota, and dysregulation of the immune system are unknown. Gut microbiota is influenced by a number of factors including diet composition and other environmental epigenetic exposures. The imbalance in gut microbiota may be ameliorated by gluten-free and dairy-free diets. Gluten-free diet increased the number of unhealthy bacteria while reducing bacterial-induced cytokine production of IL-10. Thus, gluten-free diet may influence the composition and immune function of gut microbiota and should be considered a possible environmental factor associated with immune-related disease, including nephrotic syndrome. Furthermore, the imbalance of gut microbiota may be related to the development of cow's milk protein allergy. Investigations are needed to fill the gaps in our knowledge concerning the associations between the gut microbiome, environmental exposures, epigenetics, racial influences, and the propensity for immune dysregulation with its inherent risk to the developing individual.

Successful treatment of nephrotic syndrome with a hypoallergenic diet has long been documented in several clinical studies and case reports over the past three decades. However, the use of elimination diets, such as gluten-free or dairy-free diets, in management of nephrotic syndrome has received inadequate attention from clinicians, and research on diet as a therapeutic option in nephrotic syndrome is limited.

While the mechanism is unclear, gluten-free and dairy-free diets may influence the composition and immune function of gut microbiota and decrease the risk of certain immune-mediated diseases. However, there are currently no data on the effects of altered gut microbiota on pediatric kidney disorders, such as idiopathic nephrotic syndrome. Nevertheless, new evidence has emerged in the role of gut microbiota in progressive renal disease in adults. Therefore, the aim of this review is to discuss the potential allergenic effect of dairy and gluten on nephrotic syndrome and to stimulate research on the effect of diet-induced changes in gut microbiota on nephrotic syndrome.

FOOD SENSITIVITY AND NEPHROTIC SYNDROME

The first study to investigate the link between nephrotic syndrome and cow's milk sensitivity was conducted by Sandberg *et al.* in 1977 (1). Sensitivity to cow's milk was investigated in six children ages 10–13 y old with frequently relapsing, steroid-responsive nephrotic syndrome. During the study period, prednisone was discontinued and the subjects were placed on a liquid elemental diet. Remission occurred quickly within 10 d in three of the six patients. Protein excretion decreased to less than 500 mg/24 h. The reintroduction of cow's milk resulted in significant proteinuria and edema. Four of the six subjects were able to maintain remission on a milk protein–free diet while the other two subjects experienced relapses. When prednisone 20 mg was added and the diet was restricted further to exclude cereal grains, relapses were controlled.

In 1989, Laurent *et al.* (2) investigated the connection between idiopathic nephrotic syndrome and food allergies in 26 patients ranging in age from 7 to 72 y old. The foods investigated were cow's milk, egg, chicken, beef, pork, and gluten. Subjects were given intradermal testing with various food allergens. Based on these results, patients were instructed to follow specific diet restrictions. Six of the 26 patients successfully responded to diet treatment with remission of nephrotic syndrome. Nephrotic syndrome resolved in two patients with the elimination of gluten, and in one patient with the exclusion of dairy. Three patients required the elimination of multiple foods to achieve remission (see **Table 1**).

In 1992, Sieniawska *et al.* (3) evaluated the effects of a milk-protein free diet in 17 patients, ages 1–15 y with steroid-resistant nephrotic syndrome. Of the 17 subjects, 6 responded to the milk protein–free diet with remission of nephrotic syndrome. In all six subjects, proteinuria resolved within 3–8 d of starting the diet. Full remission was achieved within 2–3 wk. Clinical signs of allergy such as eczema and bronchitis were observed in four of the six children who responded to the diet.

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Table 1 Idiopathic nephrotic syndrome (NS) and food sensitivity: summary of case reports

Study	Number of patients responding to diet therapy with remission of NS	Age range	Responsible foods	Diet method	Length of time between initiating diet therapy and remission of NS
Sandberg *et al.*	6	10–13 y	Cow's milk (4)	Elemental diet	In three patients, remission occurred within 3–10 d of starting the diet. Data N/A for three patients
			Possible gluten/wheat (2)		
Laurent *et al.*	6	7–62 y	Egg, chicken, and cow's milk (1)	Specific avoidance	Remission occurred within several weeks
			Gluten (2)		In case 1, remission occurred within 1 mo. Data N/A for case 2
			Gluten, pork, and cow's milk (1)		N/A
			Cow's milk (1)		Steroids were able to be withdrawn within 2 y of starting the diet
			Beef, pork, and egg (1)		N/A
Sieniawska *et al.*	6	1.5–3 y	Cow's milk (6)	Cow's milk protein–free diet	Remission occurred within 3–8 d of starting the diet
de Sousa *et al.*	1	5 mo old	Cow's milk (1)	Cow's milk protein–free diet	Remission occurred in 1 mo
Rasoulpour *et al.*	4	4–10 y	Cow's milk (4)	Cow's milk protein	N/A

A fourth case report by de Sousa *et al.* (4) described a 5-mo old baby girl with nephrotic syndrome due to cow's milk protein allergy. The baby was introduced to whole cow's milk early in infancy at 2.5 mo old due to poor weight gain on infant formula. The infant was hospitalized with bloody diarrhea, macroscopic hematuria and proteinuria; and treated with a milk protein–free formula (Nutramigen). Within 5 d of starting the diet, diarrhea resolved and proteinuria disappeared by 4 wk. More recently, Rasoulpour described four patients with steroid dependent nephrotic syndrome ages 4–10 y who responded to a milk protein–free diet (5).

The remission of nephrotic syndrome in response to diet strongly supports the idea that food sensitivities may trigger the disease in some cases. Despite the promising results of these studies conducted in the late 1970s to the early 1990s, very little attention has been given to diet and the potential role of the microbiome in the treatment of nephrotic syndrome over the past 20 y.

Recently, there has been a renewed interest within the nephrology community about the association between food allergy/sensitivity, particularly milk protein and gluten, with the development of nephrotic syndrome. This coincides with an increased awareness and prevalence of food allergies (6). The prevalence of food allergy has increased 18% from 1997 to 2007. In addition, children now require more time to outgrow the allergies (7,8). Although less studied in nephrotic syndrome, other dietary proteins such as gluten have been implicated in related immune-mediated conditions such as eczema. There is an increased awareness in the research community of nonceliac gluten sensitivity (NCGS) (9), and the effects of NCGS on immune function are still being elucidated. In addition to milk protein, gluten may play a role in the development of some cases of nephrotic syndrome and warrants further investigation. The effect of diet on the immune system is enormously complex and may be mediated in part by the amount and type of bacteria in the gut (10,11). The influence of diet on the gut microbiome may lead to the development of allergies or immune system dysfunction.

THE GUT MICROBIOTA

The gut microbiome is a complex community of >100 trillion microbial cells that reside in the gastrointestinal tract. It is a collection of both beneficial bacteria, which act in symbiosis with the host, and bacteria which are potentially pathogenic. Development of the infant gut microbiota is influenced by multiple factors, such as prenatal exposure, gestational age, type of delivery, and type of feeding (12). At around 1 y of age, when infants switch to regular food, the gut flora resembles the complex composition of the adult (13). In adults, the two dominant bacterial phyla are Firmicutes (Gram-positive anaerobes) and Bacteroides (Gram-negative anaerobes). Other phyla, which are present in smaller proportions, include Actinobacteria, Proteobacteria, Verrucomicrobia, Cyanobacteria, Fusobacteria, Spirochaetes, and TM7 (14).

EFFECT OF DIET ON GUT MICROBIOTA

Many studies have demonstrated that the composition of the gut microbiota is modulated by dietary habits. However, there are limited data on the effects of gluten-free or dairy-free diets on intestinal microbiome. A gluten-free diet is currently the only treatment for celiac disease, and it has been shown that the bacterial composition of the gut is altered in treated and untreated adults with celiac disease (15). However, a gluten-free diet may not completely restore the natural balance of the microbiome in those patients with gluten sensitivity. In a study by De Palma *et al.*, gluten-free diet in healthy adults led to modifications of both the composition

and immune properties of gut microbiota. Adults on gluten-free diet had a decreased number of healthy gut bacteria (*Bifidobacterium* and *Lactobacillus*) and increased number of potentially unhealthy bacteria (*Escherichia coli* and Enterobacteriaceae). However, a gluten-free diet also led to a significant reduction of bacteria-induced cytokine production (TNF-α, IFN-γ, IL-8, and IL-10) as a result of the reduction of total bacterial load (16).

EFFECT ON IMMUNE SYSTEM AND INCREASED RISK OF IMMUNE-MEDIATED DISEASE

Gut microbiota play a key role in the development of innate and adaptive immune systems. It acts on T-cell modulation, both within and outside the intestine. *In utero*, the T helper 1 (T_H1) response is suppressed to prevent inappropriate or excessive response to maternal antigens; and at birth, the neonate is skewed towards a T helper 2 (T_H2) response to novel antigens. Exposure to gut microbiota shifts this response to the development of T_H1 cells, which promotes immune tolerance and maintains a T_H1/T_H2 balance. Germ-free mice demonstrate a T-cell imbalance, with a T_H2 bias, as a result of absent intestinal microbiota. Colonization with certain bacteria, such as *Bacteroides fragilis*, can correct this T_H1/T_H2 imbalance (17,18).

Commensal bacteria also affect T_H17 cells in the colonic lamina propria (cLP) under normal and inflammatory conditions. Specific microbes are necessary for cLP T_H17 cell induction, as demonstrated in previously germ-free mice, who could only induce T_H17 cell after being colonized with a defined mixture of bacteria (19). In addition, several reports in mice have also illustrated the ability of segmented filamentous bacteria to exacerbate extraintestinal autoimmune diseases, such as arthritis, through induction of a particular pathogenic T_H17 cell (20).

Other T cells affected by gut microbiota include regulatory T (Treg) cells, which act as mediators of immunologic tolerance and homeostasis. Treg cells are important in suppressing immune responses to microbe-triggered intestinal inflammation. Therefore, loss of Treg cells by genetic mutation or antibody deletion may increase susceptibility to autoimmune and inflammatory diseases. Commensal bacteria have been shown to be essential for the development of fully functional Treg cells in a site-specific manner (18).

An altered gut microbiome, or dysbiosis, could therefore lead to altered immune functions and increased risk of disease. Failure to develop a balance between immune tolerance and active immune response is hypothesized to contribute to immune-mediated disorders. Inadequate microbial stimulation, especially in childhood, can result in impaired inflammatory T_H1 responses, which leads to enhanced T_H2 responses, and a predominance of T_H2-mediated cytokines, such as IL-4, IL-5, and IL-13 (20). These cytokines have been implicated in the development of allergic disease. Furthermore, genetic screening of the commensal bacteria has shown that reduced biodiversity in the gut is also associated with an increased risk for food allergies (21).

GUT MICROBIOTA AND KIDNEY DISEASE

There have been numerous reports, as described earlier, suggesting an association between idiopathic nephrotic syndrome and allergies, but there is weak evidence to support that nephrotic syndrome is a type of allergic disorder (22). The mechanism underlying minimal change disease, the most common cause of idiopathic nephrotic syndrome in children, is unknown but it is believed to be immune-mediated. Patients with nephrotic syndrome may have increased serum IgE levels, which may be due to increased levels of IL-13. Recent studies suggest that IL-13 may mediate proteinuria in patients with nephrotic syndrome because of its ability to directly induce expression of CD80, a transmembrane protein involved in T-cell costimulation, on the podocyte (23,24).

While there are currently no data on the increased risk of nephrotic syndrome due to an altered gut microbiota, difficult-to-treat nephrotic syndrome (steroid-resistant, steroid-dependent, or frequently relapsing nephrotic syndrome) can evolve into chronic kidney disease (CKD). There is emerging evidence in the role of the gut microbiome in the progression of CKD and its associated complications. Dysbiosis and impaired intestinal barrier function in patients with CKD have been associated with accumulation of gut-derived uremic toxins leading to insulin resistance, protein energy wasting, immune dysregulation, and atherosclerosis (25). Uremic patients show increased aerobic and anaerobic organisms in the duodenum and jejunum; patients with CKD have decreased amount of Lactobacillaceae and Prevotellaceae; and hemodialysis patients have 100 times more aerobic bacteria (Enterobacteria and Enterococci species) (26–28). This intestinal dysbiosis may be due to several factors, including uremia. Declining renal function results in urea secretion in the gastrointestinal tract. Subsequent hydrolysis of urea leads to high levels of ammonia, which could alter the growth of gut bacteria. Other contributing factors to dysbiosisin CKD patients include decreased intake of dietary fiber, frequent use of antibiotics, intestinal wall edema, slow colonic transit, and metabolic acidosis (25).

Certain gut bacteria can also generate other uremic toxins, which are normally cleared by the kidney. As kidney function declines, concentrations of metabolites, such as indoxyl sulfate, increase and cause further damage to the kidney (29). With microbial dysbiosis, overgrowth of pathogenic bacteria, and disrupted intestinal epithelial barrier, translocation of bacteria triggers a potentially harmful inflammatory response by secreting IL-1 and IL-6, promoting T_H1 and T_H17 response. Dysregulated immune response and chronic production of inflammatory cytokines lead to systemic inflammation, which could worsen the progression of CKD and development of cardiovascular disease (25).

CONCLUSION

More studies are needed to address the potential of using a dietary intervention, such as gluten-free or dairy-free diets, and avoiding potentially harmful and toxic immunosuppressive medications in children with difficult-to-treat nephrotic syndrome. Understanding how extrinsic factors such as diet

alter disease susceptibility through changes in the gut microbiome could also provide insight into novel therapeutic strategies, including interventions aimed at establishing gut symbiosis. Further investigations are necessary to fill the gaps in our knowledge concerning the associations between the gut microbiota, environmental exposures, epigenetics, and the propensity for immune dysregulation with its inherent risk to the developing individual.

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