Dear Sir,

Membranous nephropathy (MN) is regarded as a prototype of immune complex-mediated glomerulonephritis. While MN is the most frequent cause of adult idiopathic nephrotic syndrome, in most cases the causative antigens are not known. In some cases, MN has been deemed to be secondary change to specific diseases or agents. According to the literature, certain chemicals can cause MN [1-7]. This report examines a case of MN which was repeatedly exacerbated after the patient had used a 1% P-phenylene-diamine (PPD) solution as a hair dye. PPD is a main component of hair dyes. Due to its biochemical characteristics, PPD may be a factor in the pathogenesis of MN, although it is also possible that factors other than PPD may also cause MN.

A 57-year-old female was admitted for a thorough examination for proteinuria after a 2-year follow-up at a clinic. Although there was a maternal history of hypertension, there was no family history of nephrotic syndrome. The patient was engaged in doing office work and had never been exposed to toxic industrial chemicals. In addition, she had never received any drugs known to cause MN. Proteinuria and hypercholesteremia were first diagnosed during a routine examination conducted 18 months prior to her admission. At the first visit to our hospital, she was normotensive and showed no signs of peripheral edema, nor did she suffer from any common cold-like symptoms. At 12, 9 and 2 months previous to her admission, she experienced an exacerbation of proteinuria although there was no weight gain, edema, or hypertension. Her serum albumin was within the normal range. Proteinuria always disappeared within 2 months without any treatment.

Upon admission, her blood pressure was 140/80 mm Hg, and pulse was 72/min and regular. She was 158 cm tall, and weighed 56 kg. There was no evidence of arthritis, peripheral edema or eruption, no lympho-adenopathy in the cervical area, or enlargement of the thyroid gland. The thorax was clear to P&A and her abdomen was soft and flat. Her hair dyed black. The urine showed (+++) for protein and (+) for occult blood. Urinary sediment contained 2-3 red blood cells per high-power field. Her urinary protein was 0.23 g/day and creatinine clearance was 80 ml/min. The results of all hematological examinations were normal. Blood chemistry tests revealed the following: total protein 7 g/dl (70 g/l), albumin 3.4 g/dl (34 g/l), total cholesterol 274 mg/dl (2.74 g/l), serum urea 2.5 mmol/l (7.0 mg/dl), and serum creatinine 35.4 µmol/l (0.4 mg/dl). Liver function and electrolytes were normal. The ASO titer was negative as was a test for antinuclear antibodies. The complements and CH50 were within normal limits. Immune complexes (IC) were not found in the serum. Tubular function...
as represented by NAG, β2-MG, α′-MG, FENa was normal, and a 75-gram OGTT showed a normal pattern. Surface antigens for hepatitis B virus and antibodies for hepatitis C were not present. Thyroid function was within the normal range. Neither TGHA nor MCHA were detected. A gastrointestinal series and colon fiberoscopy disclosed no abnormal findings which could suggest malignancy. An electron microscopic examination of the kidney revealed that the capillary wall was mildly thickened and the presence of numerous subepithelial electron-dense deposits (fig. 1). The density of some of these deposits was reduced. These findings were compatible with stage IV MN. Idiopathic MN was diagnosed.

We monitored the patient without administering medication since she showed no symptoms of protein loss and her renal function was normal. Six months later, the fifth incidence of exacerbated proteinuria was diagnosed when she came to the hospital for a follow-up. This was 20 days after she had dyed her hair after not having done so for 6 months. During a 5-year period, she had habitually dyed her hair herself once every few months. Since her first visit to our hospital, she experienced an exacerbation of proteinuria five times. Two of these episodes occurred within 30 days of her using a 1 % PPD solution as a hair dye. The timing of the other three episodes could not be confirmed in relation to the use of hair dye. Since she has discontinued dyeing her hair, she has not experienced an exacerbation of proteinuria during the past 14 months.

In this case, no antigens nor agents known to cause MN were found. Although this case could be considered as a usual case of MN, we propose that PPD may have been a factor in the pathogenesis of MN. The first reason for this is that the intervals between hair dyeing related to the cycle exacerbation of the proteinuria. The second is that PPD, due to its biochemical characteristics, may have acted as an exogenous cationic antigen predisposing the patient to MN. The last is that our patient experienced an exacerbation of MN more than once after dyeing her hair.

PPD, a main component in hair dyes, is the most widely used hydrocarbon in the cosmetic trade for oxidizing hair dyes [8]. It is known that PPD is immunogenic in humans. Experimentally, PPD produces ana-phylactic reactions in guinea pigs. In the case of humans, an allergic reaction to PPD is usually manifested as contact dermatitis, although urticaria and asthma have also been reported in fur dyers [9, 10].

PPD, C6H4-(NH2)2, is one of the aromatic amines. Its molecular weight is 108.15 and is too low to be immunogenetic by itself. PPDs have both hydrophobic sites as a hydrocarbon and hydrophilic sites as an amine residue. Hydrophobic sites cause PPD to bind easily to structural proteins. It is supposed that PPD acquire immunogenicity as a hapten. Antigen charge is an important factor in the pathogenesis of IC-mediated glomerulonephritis. Experimental studies have shown that exogenous cationic antigens are able to bind with GBM, and that they induce in situ immune complex formations [11].
The hydrophilic site of PPD is supposed to play an important role in predisposing it to in situ IC formation. Because, at a pH of 7.4, PPD takes the form of a cation which results from the electron sink phenomenon of the nitrogen contained in the amine residue [12], PPD could acquire an affinity for GBM. The negatively charged components in GBM might permit PPD to bind directly onto the surface of endothelial and epithelial cells, causing initiation of MN via in situ IC formation.

Although it is well known that PPD causes toxic effects in humans, in this case, we believe that MN resulted from the immunogenicity rather than the toxicity of PPD. If PPD harms a specific organ, proteinuria may appear followed by other symptoms or abnormal laboratory examination findings in the affected organ. In this case, proteinuria was exacerbated solely after the use of hair dye. The tissue distribution study of PPD revealed neither localization in target organs nor prolonged retention. PPD is absorbed quickly from the skin. About 70% of administered PPD is excreted in the urine, while 30% of it is acetylated in the liver. The remainder in the body is scarcely detectable. Acute toxic effects have been reported in rats (LD50 80-98 mg/kg) with long-term topical applications [8, 13]. Our patient used about 400 mg of PPD for a single application. Small amounts of PPD used for hair dyeing once every 2 months suggest a lower probability of toxic reactions.

Most organic solvents pass through the GBM without being trapped since they are charge-free small molecules. They are mainly absorbed from tubules, and are concentrated there. Tubular dysfunction or destruction are well-known acute or subacute toxic effects of organic solvents [3, 4]. PPD is reported to cause tubular necrosis due to myoglobinuria resulting from rhabdomyolysis in acute toxic cases [14]. The aromatic structure contained in PPD is supposed to make PPD easily reabsorbed and concentrated from the tubules, while PPD acts as a cation due to the amine residue. When PPD is taken in such large amounts that toxic effects appear, not only glomerular but also tubular dysfunction may manifest. In our case, tubular function, represented by NAG, β2-MG, α1-MG, and FENa, was normal. Dysfunction localized in the glomeruli is one of the negative bases of PPD toxicity.

The clinical course of our case supports the assumption that PPD may play a causal role in the pathogenesis of MN. Although many epidemiological data and case reports suggest that repeated exposure to organic solvents is associated with a wide spectrum of renal diseases, including tubular necrosis, no adequate proof is as yet available [3, 4]. Solvent-induced MN has already been reported [2]. The case reports of familial occurrence of solvent-induced renal damage point to an immunoresponsive gene behind these diseases [6, 7]. A strong association of MN with HLA-DR3 has also been observed [15]. The number of patients with MN is minute when compared to the very large number of users of hair dye in Japan. This fact leads us to believe that renal susceptibility to PPD may be inherited. Experimentally, it has been shown that certain aromatic hydrocarbons affect immune function by binding to genetically controlled cytosolic receptors, leading to the induction of the cytochrome P450 isozyme [4]. It is not known whether PPD alters immune function as autoimmune components were not found in our case. It is interesting to speculate as to whether PPD affects immune function predisposing an individual to MN.

There are still many problems to be solved. For example, we need to determine whether other components of hair dye or metabolites of PPD may cause nephrotoxic effects. Or whether factors other than hair dye might be involved in the pathogenesis of MN. Further investigations are needed to establish an association between chronic exposure to PPD and MN.
References

Membranous Nephropathy Exacerbated after Use of Hair Dye
Nephron 1996;74:449-451