

Hemodialysis-Related Amyloidosis: Is It Still Relevant?

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ABSTRACT:

Accumulation of amyloid fibrils from β_2 -microglobulin (β_2 M) was first recognized as a characteristic osteoarticular complication in long-term hemodialysis (HD) patients and called “HD-related amyloidosis” (HRA). However, this syndrome can also be observed in end-stage renal diseases (ESRD) patients undergoing peritoneal dialysis, and even in patients with chronic renal failure *before* the initiation of dialytic therapy, suggesting that HD is not a direct cause but that accumulation of β_2 M or some β_2 M-associated molecules in the body is a common pathogenesis. Currently the term “dialysis-related amyloidosis” (DRA) is widely used for β_2 M-amyloid ($A\beta_2$ M) amyloidosis associated with ESRD, though DRA patients consist mostly of those undergoing long-term HD.

Factors other than β_2 M accumulation also play a role in the formation/local deposition of $A\beta_2$ M and disruption of tissue architecture. Conformational changes of β_2 M by misfolding/unfolding, and promoting/inhibitory effects induced by other co-existing molecules, advanced glycation and oxidation, and direct cell toxicity have also been documented.

Two technological improvements of HD have been the keys to prevent the development and progression of DRA: the efficient removal of β_2 M by using high-flux membranes, high-volume convection and adsorptive column/membrane, as well as the use of biocompatible membranes and

dialysates (e.g. ultrapure and acetate-free dialysates) which have the minimized both inflammation and β 2M production.

Epidemiologically, a decrease in the incidence of DRA has recently been reported; however, longer survival of HD patients may contribute to the development of more DRA, though with a delayed onset. In this article, we describe the pathogenesis of DRA, the strategies developed for its prevention and minimization, and the favorable epidemiological data achieved by these efforts.

Introduction

Hemodialysis (HD)/dialysis-related amyloidosis (HRA/DRA) is a systemic amyloidosis that is mainly associated with osteoarticular lesions, including carpal tunnel syndrome (CTS), trigger finger (TF), spinal canal stenosis (SCS), destructive spondyloarthropathy (DSA), joint arthropathy and bone cysts. In 1975, Warren and Otieno reported a high incidence of CTS in HD patients (1); then, in 1980, amyloid deposition was recognized as being associated with CTS (2). In 1984, Kuntz et al reported DSA in HD patients (3). In 1985, Charra et al reported shoulder pain with amyloid deposition as well as CTS in long-term HD patients (4); in the same year, beta2-microglobulin (β 2M) was identified as the precursor protein of amyloid by Gejyo *et al* (5).

Although β 2M-amyloidosis ($A\beta$ 2M) had been observed in patients undergoing peritoneal dialysis, and even in patients with chronic renal failure before the initiation of dialytic therapy (6-8), HRA was recognized as a characteristic complication of long-term HD in the earlier era of HD which used low-flux membranes (i.e., cellulosic membranes such as cuprophan) with low biocompatibility of both the membrane and the dialysate. Currently the term DRA is widely used for all $A\beta$ 2M associated with end-stage renal diseases (ESRD), even though almost all DRA patients do undergo long-term HD. The removal of β 2M from the body is almost wholly dependent on kidney function (5); therefore, greatly elevated circulating β 2M in ESRD patients with disrupted kidney function is a prerequisite for developing DRA.

Over the last two decades, even though the reduction of serum β 2M level has not reached ideal levels, technological improvement of HD (i.e., high-flux membrane, high-volume convection and ultrapure dialysate (UPD))

has certainly contributed to delay the onset of DRA and reduced its incidence and prevalence (9,10) (Fig. 1), which may in turn explain the observation that the most certain cause of DRA onset is a long dialysis vintage (10-12).

In this article, we describe the pathogenesis of DRA, and the evidence of strategies (i.e., technical improvements in HD) that have succeeded in reducing DRA from the past up to today. We then discuss the current epidemiological data and factors associated with DRA.

Pathogenesis of DRA: retention of β 2M and amyloid fibril formation

β 2M forms the beta chain of human leukocyte antigen (HLA) on all nucleated cells and is the causative protein of HRA/DRA. The production rate of β 2M is constant, but increases in inflammation, infection and lymphoproliferative disorders. Most β 2M can be removed by glomerular filtration and subsequent reabsorption and catabolism by proximal tubules. β 2M accumulates when the removal rate falls below the production rate in ESRD patients, with serum β 2M levels increasing up to 60-fold (5).

Factors other than a high circulating level of β 2M also play a role in the formation/local deposition of A β 2M and disruption of tissue architecture. In *in vitro* studies, nucleation-dependent polymerization followed by an elongation process is required for A β 2M formation. Conformational change by unfolding/misfolding of β 2M is considered a first rate-determining step. Motomiya et al reported that β 2M fragments having C-terminal unfolding, including a fragment lacking the first six amino acids (Δ N6 β 2M) (13), which has been recognized as an important amyloidogenic form, are essential for amyloidogenesis (14). Hereditary systemic amyloidosis due to the Asp76Asn- β 2M variant—a new type of A β 2M with normal renal function—also suggests the importance of conformational change for β 2M amyloidogenesis (15). Naiki and coworkers (16,17), and other researchers (18) identified numerous promoters (i.e., type 1,2 collagen, glycosaminoglycan including heparin, proteoglycan, apolipoprotein E, lysophospholipid, free fatty acids and pentraxins (serum amyloid P component and C-reactive protein) and inhibitors (i.e., α 2 macroglobulin and haptoglobin)) of A β 2M formation and elongation in *in vitro* studies.

For tissue damage caused by A β 2M deposition, advanced glycation end-products and advanced oxidation protein products, partially co-located on A β 2M in uremic states, have been reported to induce proinflammatory effects; these are assumed to be triggered by bio-incompatible dialysis and contaminated dialysate. Recently, a new mechanism of direct cytotoxicity, by which endocytosed A β 2M induced necrosis and apoptosis by disrupting endosomal/lysosomal alteration of membranes in synovial fibroblasts, was reported (19).

Extracorporeal β 2M removal in hemodialysis patients

In the first two decades after HRA/DRA was first recognized in 1980, substantial technological improvements of HD in both permeability and biocompatibility of membranes and convection were made, especially in terms of satisfactory removal of circulating β 2M. However, in the current era, the reduction of serum β 2M has still not reached ideal levels. Renal transplantation is the ultimate option for bringing serum β 2M close to a normal range with dramatic improvement of DRA symptoms, although radiologic (bone cysts) and histologic lesions do not disappear after long-term follow-up (20).

High-flux membrane

Unmodified cellulosic membranes, such as cuprophane which is both impermeable and has low adsorptivity for β 2M, have been recognized as a major direct culprit of HRA development. By contrast, high-flux membranes remove β 2M at high rates and reduce early DRA onset in HD patients (12, 21-24).

Membrane biocompatibility is assumed to be brought about by the adsorption characteristics of its materials. Indeed, adsorbed β 2M amounts vary considerably depending on the material: namely, polymethylmethacrylate (PMMA) > polyacrylonitrile (PAN/AN69) > polysulfone (PS), polyester-polymer-alloy (PEPA) and cellulose-triacetate (CTA) (24). However, judging from the remarkable difference of β 2M clearance between high-cut-off (cutoff 50–60 kDa) HD and conventional high-flow (cutoff 15–20 kDa) HD (25), the differences in sieving seem to be even more important for removal rates.

Convective therapy

In a retrospective study of more than 6000 individuals with a median

observation period of about 2 years, Locatelli et al documented the superiority of convection (i.e., hemofiltration (HF) and hemodiafiltration (HDF)) compared to diffusion (i.e. conventional HD) in decreasing DRA morbidity and delaying the need for CTS surgery (26). Currently, several convective modalities (i.e., pre-/post-/mid- dilution HDF, on-line-/off-line HDF, pull and push HDF, double high-flux HDF) are available; additionally, high-flux HD and intermittent infusion HDF can be considered forms of “low-volume HDF”, in which convective exchange occurs via internal back-filtration within the dialyzer. All these modalities can remove β 2M with great success compared with low-flux HD. Thus, the major determinants of β 2M removal per session are membrane permeability, duration of treatment, and convective volume (27). Furthermore, the effective lowering of serum β 2M levels by HDF as compared with low-flux HD is more prominent in patients without (vs. with) residual renal function (28).

In recent decades, the targets of convective therapies have shifted to the removal of small proteins or protein-binding uremic toxins which are larger than β 2M. Several randomized control trials (RCTs) studying, e.g., high-flux versus low-flux (29-31) HD, HDF versus HD, and higher- versus lower-volume HDF, have shown certain favorable results for convective therapies and their higher volume in preventing erythropoietin-stimulating agent resistance, endothelial dysfunction, insufficient management of serum phosphate, intradialytic hypotension (32-34), as well as in reducing cardiovascular events/death and improving survival (35-40); meanwhile, there are no noteworthy prospective RCTs designed to examine the effect of convective therapies on the outcome of DRA.

A few postmortem studies have shown that A β 2M deposits in long-term HD patients are not specific to osteoarticular tissue; the main other organs involved have been the vascular walls, the heart, gastrointestinal tract and lungs (41). In the latest version of amyloid classification by the Nomenclature Committee of the International Society of Amyloidosis, the type of A β 2M associated with DRA was modified from ‘localized’ to ‘systemic’ (42).

The hypothesis that β 2M deposition, particularly in the blood vessels and heart, would also incite specific processes with pathogenic effects in the

absence of extensive A β 2M deposition was suggested in a review article (27). Indeed, this hypothesis explains the fact of favorable cardiovascular outcomes of higher convective therapy in patients even with relatively short dialysis periods compared to that of DRA onset. On the other hand, Uji et al demonstrated that a few intermediate forms of β 2M, which were revealed by capillary electrophoresis, returned from the tissues and increased in the serum even after the use of online HDF treatments, suggesting that amyloidogenic β 2M forms still cannot be removed by the current higher convective therapy (43).

Intensive therapy

Intensive regimens including nocturnal HD (8 hours, six night/week) and daily hemofiltration with high-flux membranes can remove 5- to 8-fold more β 2M than conventional HD and decrease serum β 2M levels significantly. Nevertheless, serum levels in these treatments do not reach the normal range (44, 45). Although a recent meta-analysis showed lower systolic blood pressure, increased hemoglobin levels and lower serum phosphate level in patients undergoing nocturnal HD compared with those undergoing conventional HD (46), there have been no RCTs examining the effect of intensive therapy on DRA outcomes.

β 2M-adsorptive apheresis

A β 2M-adsorbent column, Lixelle, which connects to a dialyzer in tandem for direct hemoperfusion for DRA, became commercially available in Japan in 1996, in Europe in 2013 and in USA in 2015. The inside of the column is composed of porous cellulose beads with a hydrophobic hexadecyl chain as a ligand. The determinants of the adsorption are both size and hydrophobicity; therefore, any hydrophobic molecule weighing 4,000–20,000 g/mol can be adsorbed in the column; indeed, β 2M (molecular weight 11,800 g/mol) is highly adsorbed. The column adsorbs various other molecules as well as β 2M: numerous inflammatory cytokines (47), microbial fragments such as endotoxins and peptidoglycans, free light chains, myoglobin, bilirubin and drugs (digoxin, antibiotic agents); therefore, a synergistic effect of β 2M elimination and anti-inflammatory effects is assumed.

Treatments with Lixelle have been reported to be effective for improving both subjective symptoms, including joint pains and stiffness and activities

of daily living (48-52), and objective findings such as pinch strength and the median motor terminal latency (48). Furthermore, decrease or arrest of bone cyst formation with significant β 2M clearance was also reported (49, 51); however, these studies were conducted in small numbers of patients and were evaluated at an early point within short-period treatments of 1–2 years. Gejyo reported the results of a Japanese nationwide questionnaire survey from 345 patients who had used Lixelle; the mean durations of dialysis and Lixelle treatment were 25.9 ± 6.2 years and 3.5 ± 2.7 years, respectively. Based on self-evaluation by patients, the worsening of symptoms was inhibited in 84.9–96.5%, suggesting that Lixelle treatment improved symptoms or prevented the progression of DRA in most patients (52).

In the health insurance system in Japan, Lixelle cannot be used with HDF. From several case reports in Japan, even though the β 2M removal rate was equivalent, HD with Lixelle was superior to convective therapy including online HDF and pull and push HDF in relieving joint pain. There are no RCTs comparing the effect of Lixelle with that of higher convective therapy; however, there may be differences in clinical indication between the two therapies. According to previous reports and expert opinions, Lixelle should be used in patients with severe joint symptoms, and/or in patients who have contraindications for higher convective therapy, such as those who are elderly, have malnutrition or chronic inflammation. In the latter case, the usage of low-flux and biocompatible membranes (i.e., PMMA and AN) are recommended.

Prevention of inflammation and β 2M production during hemodialysis

Membrane biocompatibility

Bioincompatible membranes such as cuprophane cause complement activation, followed by gene expression of inflammatory cytokines in peripheral blood cells (53). Evidence from both *in vitro* and *in vivo* studies suggests that cuprophane induces excessive β 2M production(54, 55). Although these findings are not consistent (56), low-flux cuprophane membranes are the most permeable to pyrogens from dialysate (57), which stimulates inflammation, leading to excessive β 2M production.

Dialysate

Regarding the biocompatibility of the dialysate, elimination of acetate and sterilization have been two important issues. Acetate-containing dialysate can induce inflammatory cytokine production, peripheral vasodilatation, and suppression of myocardial function, resulting in intradialytic hypotension in HD patients. Several small-scale RCTs have been conducted and have discussed these symptoms, although their conclusions varied. Metabolic acidosis and higher plasma β 2M levels were observed in HD patients using acetate-containing dialysate compared with bicarbonate-containing dialysate. Metabolic acidosis may be relevant to DRA. In normal men, NH_4Cl loading increases β 2M mRNA expression in lymphocytes (58).

The use of ultrapure dialysate (UPD), one with almost no bacteria or endotoxins, as an important issue in the current era of highly convective therapy. In 226 long-term HD patients with cellulosic membranes, the prevalence of CTS onset was significantly lower in patients with UPD compared with non-UPD (59). Several studies have also demonstrated that the use of UPD correlates with a decrease of DRA (23,60). A recent meta-analysis concluded that the use of UPD in HD patients decreased markers of inflammation and oxidative stress, increased serum albumin and hemoglobin and decreased erythropoietin requirement (61); however, there is no information on DRA onset in this study.

Current epidemiology of DRA

There are two reports demonstrating that the prevalence of DRA has considerably decreased (9,10). In a single-center study from Germany in 1997, Schwalbe et al reported the prevalence of DRA in 1988 versus 1996 (9); randomly selected patients studied in 1988 were matched by time on HD (mean 71 months, range 3 to 207) and age (mean 51 years, range 22 to 80) with patients of the 1996 population. Compared to the 1988 population, the 1996 population exhibited significantly lower prevalence of DRA in both CTS (1/43 vs 7/43) and bone cysts (positive patients, 3/43 vs. 13/43; possible sites, 7/272 vs 33/272), and less home HD and more frequent usage of reverse osmosis water with bicarbonate buffer than acetate for dialysate preparation. No difference in serum β 2M levels between the two groups was evident, and the proportion of time occupied by high-flux synthetic membranes usage in total dialysis time was quite low in both groups (13%

of 1996 and 6% of 1988). The author speculated that β 2M removal is unlikely to be the source of the remarkable decrease of DRA, and that other factors, for example, dialysate composition and purity, may be involved.

Recently, we documented the updated epidemiology of DRA based on the national registry data from Japanese Society for Dialysis Therapy (JSDT) (10). We retrospectively compared the incidence of first-time CTS surgery as a proxy for DRA onset in 1998 (n=36,489) versus 2010 (n=166,237) cohorts (Fig. 1). The incidence of first-time CTS surgery was significantly higher in the 1998 cohort than in the 2010 cohort (1.77% versus 1.30%, adjusted OR 2.22 (1.68-2.95)). This favorable result of the 2010 cohort compared with the 1998 cohort was most prominent in patients with longer dialysis periods, younger age and lower pre-HD β 2M (Fig. 2). Using this 2010 cohort, we showed that other factors associated with CTS besides dialysis duration were age, gender, serum albumin, and diabetic nephropathy. We also indicated that β 2M clearance >80% may contribute to decrease the incidence of CTS (62) (Fig. 3).

According to the latest Japanese national registry data from JSDT at the end of 2016, the prevalence of dialysis patients who used Lixelle or another adsorptive dialyzer (i.e., AN69 membranes) was only 0.43% (1,355/318,814). However, as dialysis vintage increased, the value increased; 1.8 (230/12,484), 4.1 (284/6,897), 8.0 (317/3,987), 10.3 (222/2,152), and 11.5% (91/793) for dialysis vintage of 20-25, 25-30, 30-35, 35-40, and \geq 40 years, respectively.

Although long-term survival of HD patients may increase the incidence of delayed onset DRA, the technological improvement of HD certainly has decreased the incidence of early onset DRA.

Conclusions

The great success of HD in removing β 2M (or some β 2M-associated molecules) seems to be a major contributor to the delay of DRA onset, but this is still a controversial hypothesis. Again, DRA is a disease that develops only in ESRD patients in whom β 2M has accumulated remarkably in the body. Even if serum β 2M levels cannot be lowered, better removal of β 2M can inhibit irreversible deposition into tissues by maintaining a dynamic equilibrium between the serum and tissue. Additionally, the improved biocompatibility of both membranes and

dialysates minimizes β 2M production and inflammation, which could also help prevent the development and progression of DRA.

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Figure legends

Fig. 1 Comparison of CTS new incidence rates in cohorts from 1998 and 2010 in Japan. The incidence of first-time CTS surgery was significantly higher in 1998 than in the 2010 (1.77% versus 1.30%). adapted from Hoshino J, Yamagata K: Significance of the decreased risk of dialysis-related amyloidosis now proven by results from Japanese nationwide surveys in 1998 and 2010. *Nephrol Dial Transplant*. 2016; 31: 595-602 (reference 10). Used with permission of the ERA-EDTA

Fig. 2 Adjusted OR of CTS onset in the two populations in 1998 and 2010, by dialysis duration (A), age (B), pretreatment serum β_2m (C) and β_2m clearance (D). ORs were adjusted by dialysis duration, age, sex, primary kidney disease, modality, body mass index, albumin, CRP, Kt/V, normalized PCR and β_2m clearance. In the analysis including pre-dialysis β_2m , β_2m removal was used instead of β_2m clearance. adapted from Hoshino J, Yamagata K: Significance of the decreased risk of dialysis-related amyloidosis now proven by results from Japanese nationwide surveys in 1998 and 2010. *Nephrol Dial Transplant*. 2016; 31: 595-602 (reference 10). Used with permission of the ERA-EDTA

Fig. 3 Adjusted ORs of CTS onset. ORs were adjusted by dialysis duration, age, gender, primary kidney disease, history of smoking, history of hypertension, dialysis modality, use of high-flux membrane, BMI, serum albumin (Alb), Kt/V, nPCR, and serum β_2MG clearance. CGN, chronic glomerulonephritis; DM, diabetic nephropathy; ADPKD, autosomal dominant polycystic kidney disease; SLE, systemic lupus erythematosus. adapted from Hoshino J, Yamagata K: Carpal tunnel

surgery as proxy for dialysis-related amyloidosis: results from the Japanese Society for Dialysis Therapy. *Am J Nephrol.* 2014; 39: 449-458 (reference 62). Used with permission of S. Karger AG

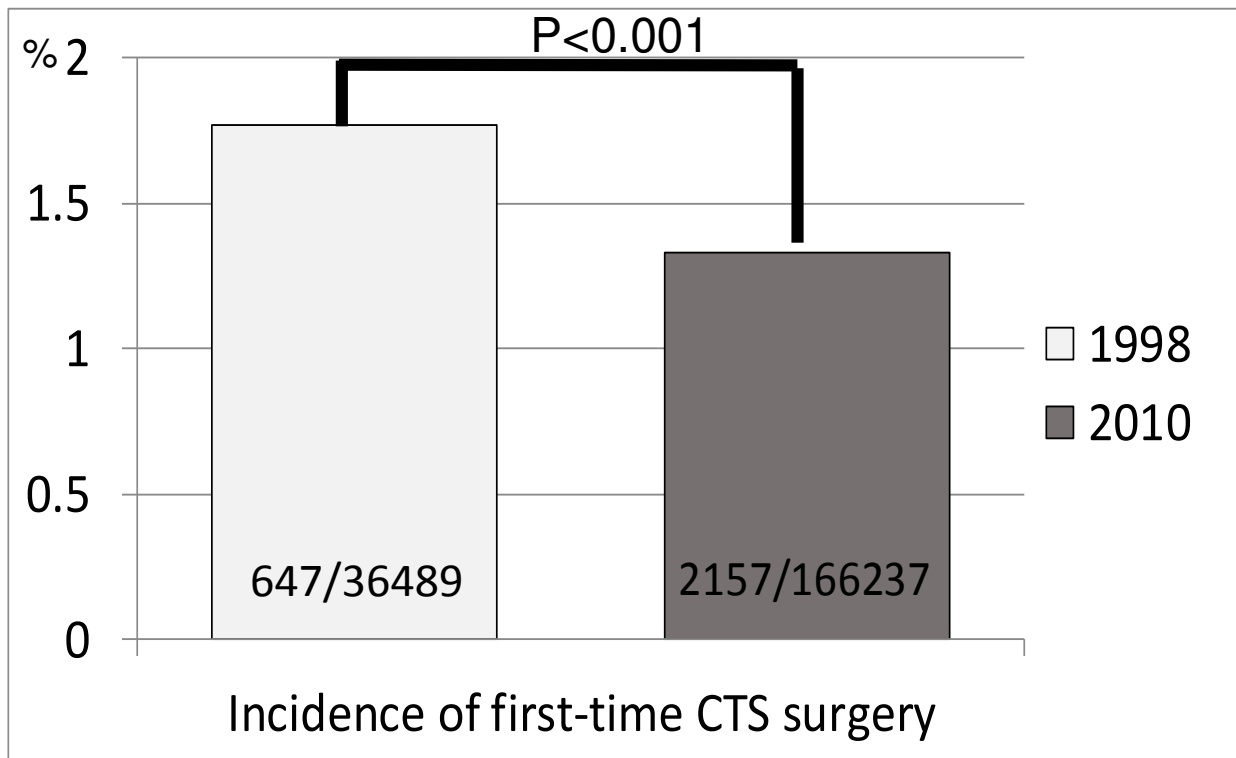


FIGURE 1

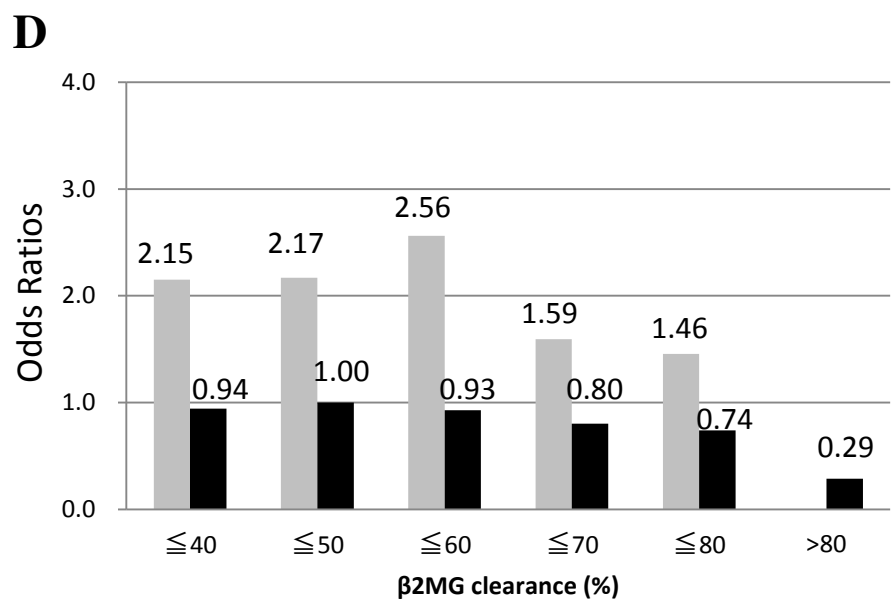
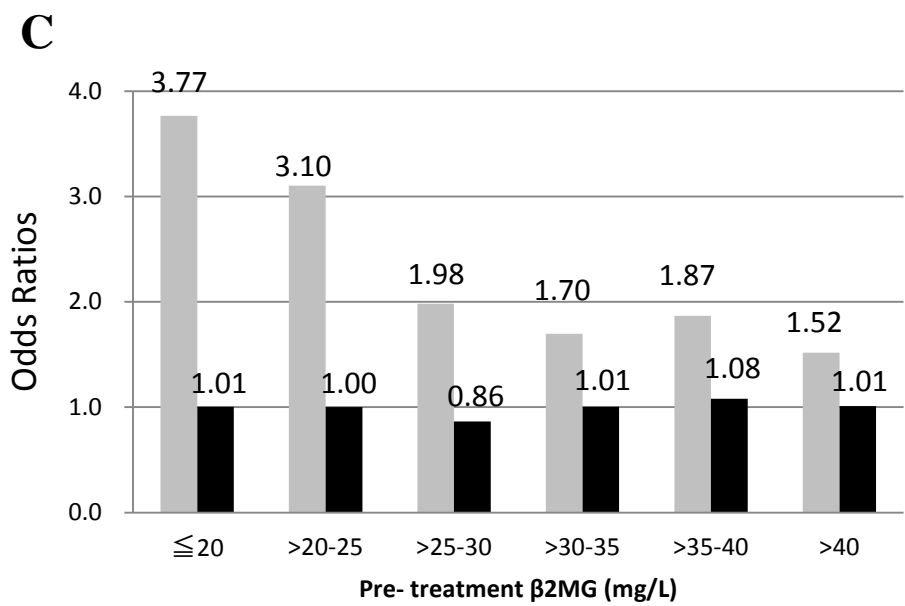
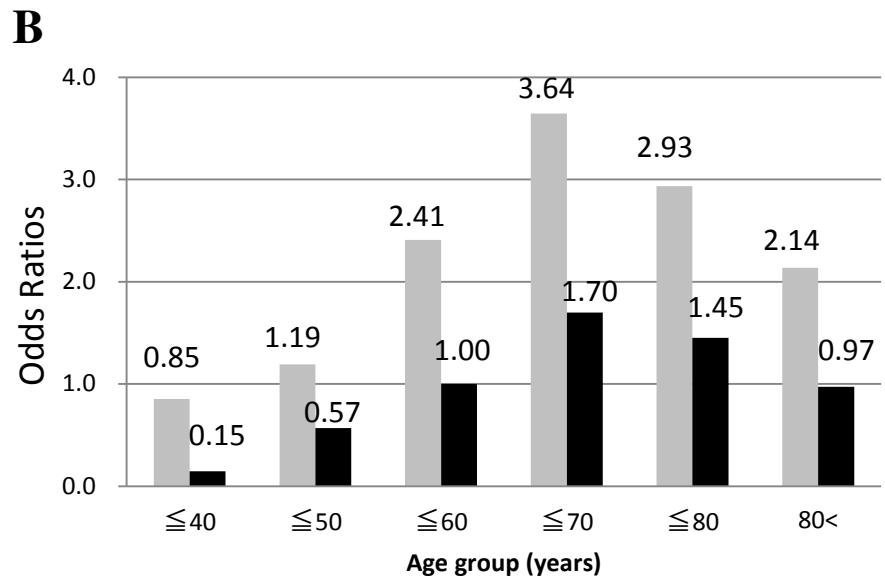
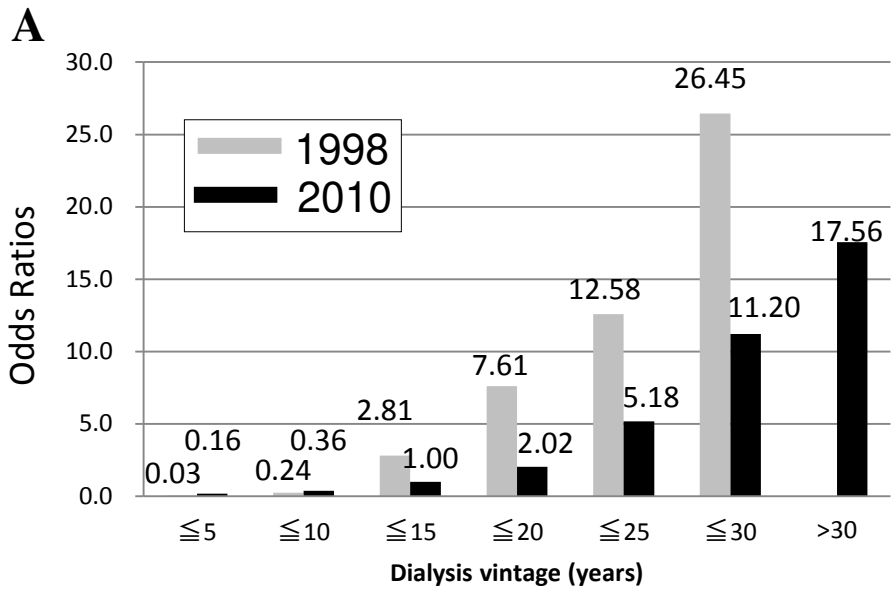


FIGURE 2

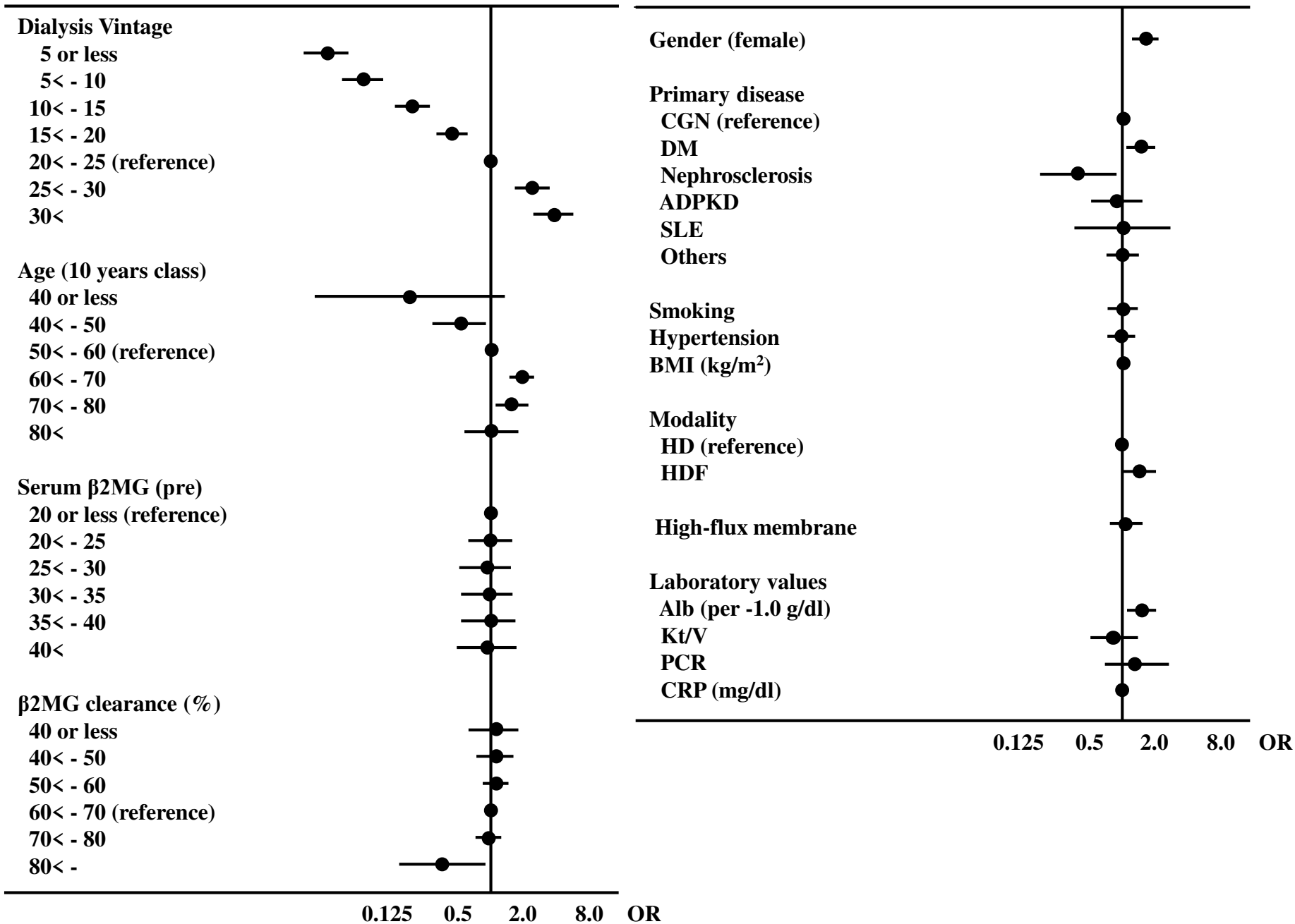


FIGURE 3