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EDITORIAL REVIEW

β_2-microglobulin: Its significance in the evaluation of renal function

β_2-microglobulin (β_2M) was isolated in 1968 by Berggård and Bearn [1] from the urine of patients with Wilson's disease and chronic cadmium poisoning, both conditions characterized primarily by proximal tubular damage. This discovery was followed by extensive investigations regarding its structure, function, metabolism and its role in the evaluation of renal function [2–4].

β_2M is a low molecular weight (LMW) protein of 11.800 daltons composed of 100 aminoacids with one disulphide bridge [5] (Fig. 1).

β_2M has been identified as the light chain of the class I major histocompatibility antigens (HLA—A, B and C) (Fig. 2). They are found on the cell surface of all nucleated cells by which they are synthesized [6]. β_2M is non-covalently attached to the heavy chain and is essential to its serological specificity. The HLA molecule loses its serological specificity when not attached to β_2M.

As a result of metabolism and degradation of HLA, β_2M is dissociated from the heavy chain and appears in its free form in the extracellular fluid. This process is called "shedding." There is also the possibility that β_2M is excreted in its free form from the cells or is released after their destruction. At least 95% of β_2M in plasma or urine is present as the free monomer, only a very small quantity being associated with other molecules [4].

Methodology of measurement

β_2M can be measured in plasma, serum, urine and other human fluids like saliva, cerebrospinal or pleural fluids. It is usually measured by radioimmunoassay or enzyme–linked immunosorbent assay (Elisa). These methods are most reliable. Other methods like gel electrophoresis, radial immunodiffusion and nephelometric assays have been described. Normal serum values are 1.1 to 2.7 mg/liter and the normal urinary excretion is < 370 μg/24 hr. There is no disagreement between investigators concerning the measurement of β_2M in serum or plasma [7–15]. The interpretation of the results of urinary β_2M determinations is sometimes a matter of discussion.

The instability of urinary β_2M has been a matter of disagreement between different investigators. All agree that β_2M is unstable at room temperature, in urine with a pH < 5.5. However, at body temperature a rapid and irreversible loss of β_2M occurs in urine with a pH < 6.0 and in urine of patients receiving gentamicin [8, 16]. Furthermore, loss of β_2M near neutral pH, caused by enzymes released from leukocytes and neutrophil granulocytes, may sometimes be found in pyuria [17]. This whole pattern of instability is probably caused by proteolytic enzymes, as these processes are inhibited by preheating the urine to 80°C or addition of low doses of specific enzyme inhibitors [16, 17].

As urine is normally stored in the human bladder for at least two hours before voiding, we advise ensuring production of urine with a pH ≥ 6.0. This can be achieved by giving alkali to the patients before and during urine collection rather than adding alkali to the urine after voiding [8].

To ensure a urinary pH ≥ 6.0, the subject should be given 4 g of sodium bicarbonate on the evening preceding the experiment, and another 4 g in four divided doses the following day, during the 24 hour collection. With these measures most urine collections have a pH of 6 or more. Only urine specimens with a pH ≥ 6.0 should be assayed.

Production and turnover of β_2-microglobulin

Turnover studies with I^{125}-labelled β_2M in humans have shown that the production in normal adults is 0.11 to 0.18 mg/hr/kg (mean 0.13 mg/hr/kg) or 150 to 200 mg β_2M daily [7]. Elimination of the protein occurs almost completely by the kidney through glomerular filtration. In normal persons, with a normal glomerular filtration rate (GFR) there is a rapid turnover of β_2M ($t\ ½ = 2.1$ hr) and serum concentration amounts to a mean of 2.0 mg/liter (range 1.0 to 2.7 mg/liter) [8].

Increased serum values in the presence of a normal GFR indicate increased β_2M production. This has been observed in lymphoproliferative diseases, autoimmune diseases like SLE, immune system associated diseases (Crohn's disease, chronic hepatitis, sarcoidosis, vasculitis), in patients with AIDS and in some malignant diseases like Hodgkin's disease, non-Hodgkin lymphoma, leukemia and in multiple myeloma [3, 4].

Renal handling of β_2-microglobulin

About 95% of all free β_2M is filtered by the normal glomerulus and subsequently almost completely reabsorbed and catabolized by the cells of the proximal tubules. β_2M enters the cells by endocytosis. The endocytic vesicles fuse with lysosomes, where the reabsorbed proteins are degraded to amino acids.

A normal kidney is able to reabsorb about 99.9% of the filtered β_2M, which means that maximally 370 μg/24 hr is excreted in the urine [3]. Malfunction of the proximal tubules with a normal GFR will be accompanied by a decreased tubular reabsorption and an increased urinary excretion of β_2M.

Intravenous infusions of ornithine, arginine and especially lysine [9, 10] cause transient tubular proteinuria, probably through temporary blocking of tubular membrane receptors. The greatly–increased protein excretion, in particular β_2M, returns to normal levels within one hour. Lysine is especially

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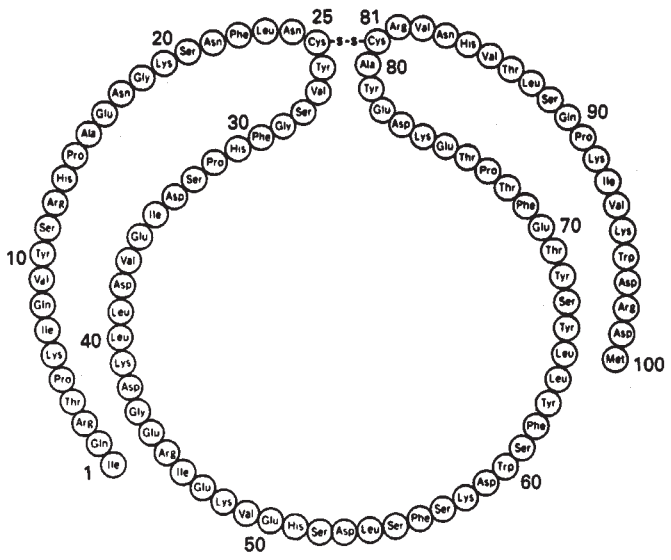

Fig. 1. *Primary structure and physicochemical characteristics of β_2-microglobulin.* From Phadedoc Diagnostic Communications 6; 4: 1979 (Pharmacia Diagnostics AB, Uppsala, Sweden).

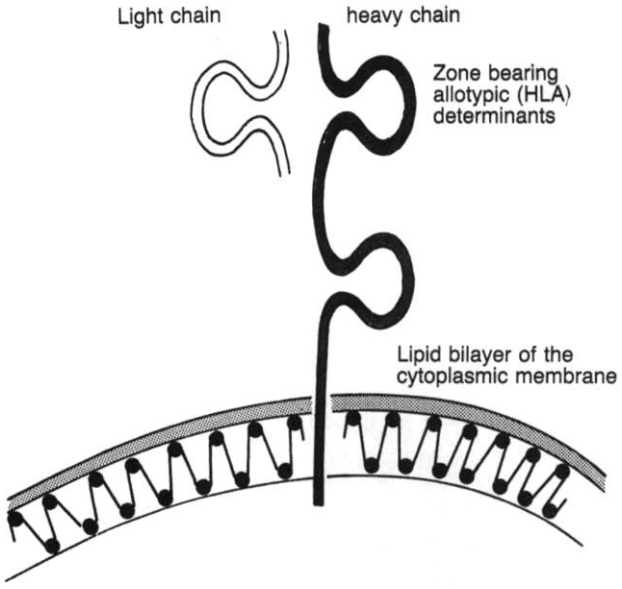

Fig. 2. *A schematic representation of the HLA complex (class I) with the heavy chain (right side) penetrating the cytoplasmic membrane and the light chain being β_2-microglobulin attached non-covalently to the heavy chain.* From Phadedoc Diagnostic Communications 6; 9: 1979 (Pharmacia Diagnostics AB, Uppsala, Sweden).

effective causing the excretion of β_2M to rise to exceptionally high values of about 60% of the amount filtered.

The circadian rhythm of urinary β_2M excretion has been studied in normal humans and in patients with the nephrotic syndrome [11]. Twelve of 20 patients had a "normal" circadian rhythm for β_2M excretion with a maximum around 15 hours and a minimum around 4 hours. The others had different excretion patterns. The day–night variability in the excretion of β_2M is also present in the absence of clinically important proteinuria. As β_2M easily passes the glomerulus, the increase in urinary excretion during daytime is probably of tubular origin [11].

β_2-microglobulin and glomerular filtration rate

As β_2M leaves the body almost exclusively through glomerular filtration, there is an inverse correlation between serum β_2M and GFR (Fig. 3); when GFR decreases both serum β_2M and serum creatinine will increase proportionally. As only about 1% of the total removal of β_2M takes place through extrarenal pathways [10, 12] serum levels of β_2M would be ideal to evaluate GFR. However in several disease states, there is an increased production of β_2M, resulting in an increased serum concentration notwithstanding a normal GFR.

β_2-microglobulin in early childhood

β_2M has been used as an indicator of renal tubular maturation in infants born from the 32nd to the 41st gestational week [13]. Serum concentration and the amount filtered increase fivefold. The urinary excretion decreased between the 32 and 35 weeks and increased again thereafter. These results and those of others [14] suggest that proximal tubular maturation lags behind glomerular function and that glomerulo-tubular balance for β_2M occurs at about 35 weeks. Fractional reabsorption of β_2M increases from 87% at 32 weeks of gestational age to 98% at 40.5 weeks, to increase further to 99.9% at 21 months of age, this being the adult value [15].

Tubular reabsorption capacity for β_2-microglobulin

Maximal tubular reabsorption of β_2M (Tm_{β_2M}) is a matter of controversy and awaits definite clarification. Human β_2M, although now available, has not yet been used in human experiments. Observations have been recorded in patients with increased endogenous production of β_2M.

Five patients with the hepatorenal syndrome with serum β_2M increased to 8.6 to 15.8 mg/liter, had no increase in urinary β_2M excretion [18]. These and other similar observations cast doubt on the postulated renal threshold of 4.5 mg/liter for β_2M suggested by Wibell and Evrin [19].

Constant infusions of human β_2M to dogs at rates ranging from 51 to 269 μg/min showed that renal extraction exceeded the rate of glomerular filtration at all levels of β_2M delivered to the kidney [20]. They concluded that β_2M is extracted from renal blood by glomerular filtration and, in addition, by a mechanism independant of GFR. No apparent saturation point for uptake of filtered β_2M or for that extracted in excess of filtration which could be demonstrated at arterial delivery rates as high as 740 μg/min.

Increased tubular β_2-microglobulin reabsorption

In sickle cell nephropathy, a condition characterized by an increased proximal tubular function, the fractional β_2M excretion was found to be decreased. Tubular reabsorption of β_2M was increased and β_2M clearances decreased [21].

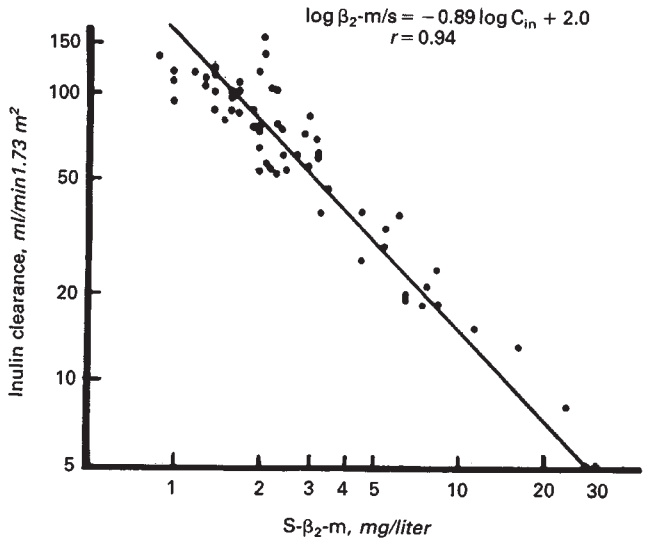

Fig. 3. *The serum levels of β_2-microglobulin versus inulin clearance in 61 subjects with normal and decreased GFR.* From [12].

Increased urinary β_2-microglobulin excretion in renal disorders

Many pathologic conditions in which the proximal renal tubule is involved, are accompanied by an increased renal excretion of β_2M as a result of diminished tubular reabsorption.

Two to 36 percent of patients treated with aminoglycosides develop signs of drug–induced renal functional derangement [22]. The damage is predominantly restricted to the proximal tubule, where the aminoglycosides accumulate, reaching concentrations that can be more than 50 times as high as the serum concentration. The result is damage to the brush border of the proximal tubules, or even their desintegration due to swelling and rupture of lysozomes, thus leading to autolysis and necrosis of the cells. An increased renal excretion of brush border and lysosomal enzymes is observed and a decreased reabsorption of β_2M, preceding the decrease in GFR and elevation of serum creatinine [22–27].

Experimental studies have revealed some of the basic pathologic mechanism in aminoglycoside nephrotoxicity [28]. Increased β_2M excretion heralds aminoglycoside induced damage before the rise in serum creatinine [22, 24]. Not all patients on aminoglycoside therapy with increased urinary β_2M will develop severe renal damage. In one study, 71% of these patients had some elevation of β_2M, but only 33% had a rise in serum creatinine [23]. Comparable observations were made by others, who observed an increase in β_2M four to five days prior to elevation in serum creatinine. Very high urinary β_2M was observed in patients with cirrhosis of the liver when treated with aminoglycosides [25].

Although most of the observations were done in patients treated with gentamicin, aminoglycoside nephrotoxicity has also been observed during treatment with tobramycin and amikacin [26]. Aminoglycosides in combination with cytoxins, especially methotrexate, appear to be excessively nephrotoxic [27, 28].

In conclusion, it can be stated that about 5 to 10% of patients treated with aminoglycosides develop serious nephrotoxic reactions, characterized primarily by damage to the proximal tubules. An extremely high excretion of β_2M (up to 50 to 100 mg daily) can be observed which precedes the decrease in GFR by about five days. This means that frequent urinary β_2M estimations can be useful in early detection of renal damage and allow discontinuation of these drugs.

In Balkan nephropathy, a chronic tubulointerstitial nephropathy of unknown etiology, with a high prevalence in some areas of Bulgaria, Romania and Yugoslavia, tubular proteinuria, especially increased urinary β_2M, is one of the first signs of the disease [29, 30]. It is therefore a suitable screening test in epidemiological studies [31].

Heavy metals

Most heavy metals accumulate in the kidney and especially in the proximal tubules. Cadmium (Cd), used in many industries, has been extensively studied in relation to its renal toxicity. Its excretion from the kidneys after exposure is very slow (10 to 34 years), which means that its effects on the kidney will be apparent for many years. The earliest sign is tubular proteinuria. Cadmium workers may have a 100- to 1,000-fold higher excretion than normals. Protein excretion was found to be 10 to 20 times higher in smokers than in non-smoking workers due to contamination of their hands [32]. Population studies in cadmium–exposed areas in Sweden, Japan and the United Kingdom showed an increased excretion of β_2M in the urine of the inhabitants, which was related to the duration of the exposure [33, 34]. In Japan the combination of renal dysfunction and osteomalacia in these patients is known as *Itai–Itai disease*. Cadmium damage of the proximal tubules is usually irreversible, although in some patients improvement has been observed after removal from exposure [35].

The renal abnormalities resulting from chronic mercury (Hg) poisoning, are comparable with those of chronic cadmium intoxication. A significant increase in renal β_2M excretion has been observed [36]. It is endemic in inhabitants around a small bay in Minamata in southern Japan. The afflicted persons had eaten large amounts of fish and shellfish that were extremely contaminated with methyl mercury discharged from industrial plants (*Minamata disease*).

Although lead and gold are known to cause kidney damage, β_2M excretion has only been incidently studied in intoxications with these heavy metals [3]. On the other hand, many observations have ben made on the nephrotoxicity of cisplatinum (cis-dichlorodiamine platinum II), a potent anti-tumor agent [37, 38]. In the majority of cases a transient rise in urinary β_2M has been observed [3, 39].

Fanconi's syndrome and other causes of tubular proteinuria

Specific tubular diseases like Fanconi's syndrome, Wilson's disease, nephrocalcinosis, untreated congenital galactosemia, chronic potassium depletion, cystinosis and other tubular diseases are characterized by an increased urinary excretion of β_2M. This is also the case with interstitial nephritis of known and unknown origin [40]. Fractional excretion (FE) of β_2M was significantly lower in children with glomerular lesions than with tubular lesions (mean 0.104% vs. 4.27%; normal < 0.36%). Of 30 children with glomerulopathies who underwent renal biopsies, 13 also had tubulointerstitial lesions. With the exception of two, all had increased FE-β_2M. Those without evidence of

tubulo-interstitial disease had normal values [41]. This means that the estimation of FE-β_2M can be helpful to diagnose tubulo-interstitial involvement in glomerulopathies.

β_2-microglobulin and radiocontrast agents

One of the major potential hazards of angiography are adverse effects on the kidneys. Radiocontrast agents may cause a decrease in renal blood flow, increase in vascular resistance or have a direct toxic effect on the kidney. All these factors may lead to a reduction in GFR, a nonselective proteinuria or sometimes acute renal failure. Risk factors are preexisting renal disease, advanced age, the volume of contrast material, the place of administration and diabetes [42, 43].

In a prospective study on 28 patients, aged 28 to 70 years who underwent coronary angiography, we were able to demonstrate a significant temporary decrease in GFR and a temporary increase of urinary alanine aminopeptidase (AAP, a brush border enzyme), β_2M and N-acetyl-β-glucosaminidase (NAG, a lysozomal enzyme from the proximal tubules) [40].

β_2-microglobulin in connective tissue diseases

Rheumatoid arthritis

In patients with rheumatoid arthritis renal disease is not uncommon. Recently, proximal tubular dysfunction was demonstrated in 24% of the patients studied [44]. Another important factor is the use of numerous potential nephrotoxic agents. Acute tubular necrosis, interstitial nephritis and analgesic nephropathy are well known complications [45]. There are several studies demonstrating the usefulness of β_2M for evaluating the toxic influence of gold and cyclosporine on the kidney tubules [46, 47].

Sjögren syndrome (SS)

For more than 20 years it has been known that renal tubular dysfunction occurs in SS. Clinical and laboratory findings vary from a decrease in concentrating capacity, to renal tubular acidosis or Fanconi syndrome. Tubulointerstitial nephritis and tubular atrophy have been observed in the majority of kidney biopsies. Our own observations employing urinary β_2M suggest that proximal tubular dysfunction occurs even in patients with primary SS, who have not received any pharmacotherapy [45].

Urinary tract infections

In hospital practice it is not always easy to diagnose or exclude pyelonephritis or to distinguish between upper and lower UTI. Methods like bladder washout and ureteral catheterization may be the most reliable, but they demand instrumentation of the urinary tract with all the hazards of an invasive technique and the danger of introduction of bacteria. The technique of fluorescence of antibody coated bacteria looked promising, but after five years experience this technique appears to be unreliable [48, 49].

From our own studies [49] β_2M appears the method of choice to distinguish between upper and lower UTI (Fig. 4). All patients with pyelonephritis had a significant increase in 24-hour urinary β_2M excretion. On the other hand, in patients with cystitis the β_2M values were completely normal. There was no overlap in 24-hour urinary β_2M values between the two groups of patients.

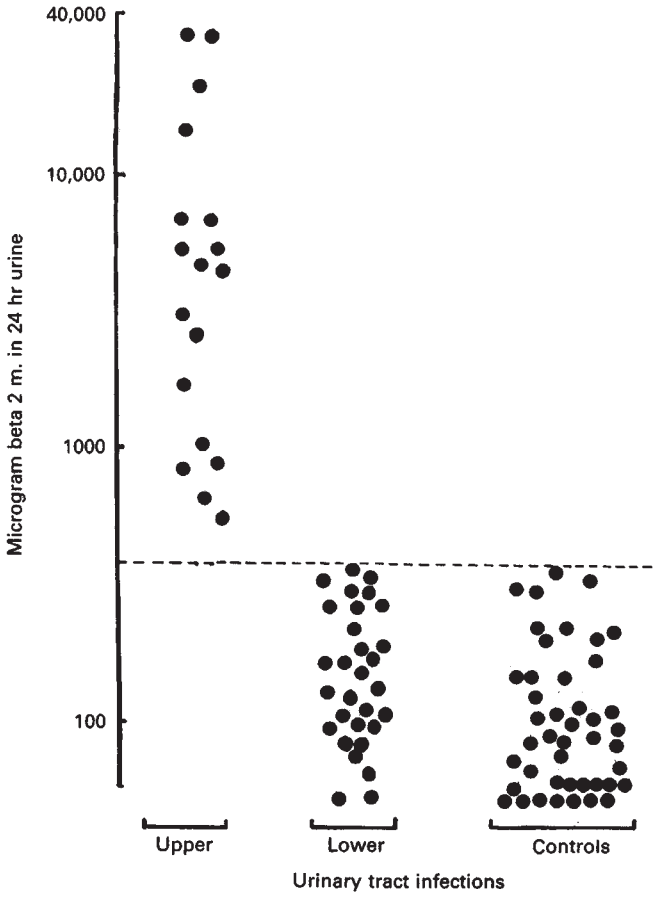

Fig. 4. *Twenty-four-hour urinary excretion of β_2-microglobulin in 18 patients with upper, 30 patients with lower urinary tract infections and 44 controls.* From [3].

Moreover, serial measurements of β_2M (Fig. 5) are useful to monitor the efficacy of therapy and the detection of recurrences in upper UTI [50]. The introduction of urinary β_2M estimations in the management of these patients could well be of great advantage in the evaluation of treatment and in the prevention of end stage, pyelonephritic renal disease.

β_2M and renal transplantation

Serum β_2M has been extensively investigated in renal transplantation [51–54]. In successful renal transplantation serum and urinary levels of β_2M decreased significantly. Frequent determinations of serum and urinary β_2M after transplantation represent a sensitive assessment of glomerular and tubular function in the transplanted kidney. There is much disagreement about the value of serum β_2M in the diagnosis of rejection.

Fields et al concluded that an increase in serum β_2M has proven to be a highly sensitive indicator of rejection (97%) and attains a specificity (84%) comparable with creatinine [53]. These investigators claim that β_2M has a distinct advantage over creatinine: it changes significantly sooner than creatinine. On the other hand Bäckman et al [54] came to the conclusion that serum β_2M does not differentiate rejection from cyclosporine toxicity, but may be helpful in the early diagnosis of cytomegalovirus infection. Some investigators consider signif-

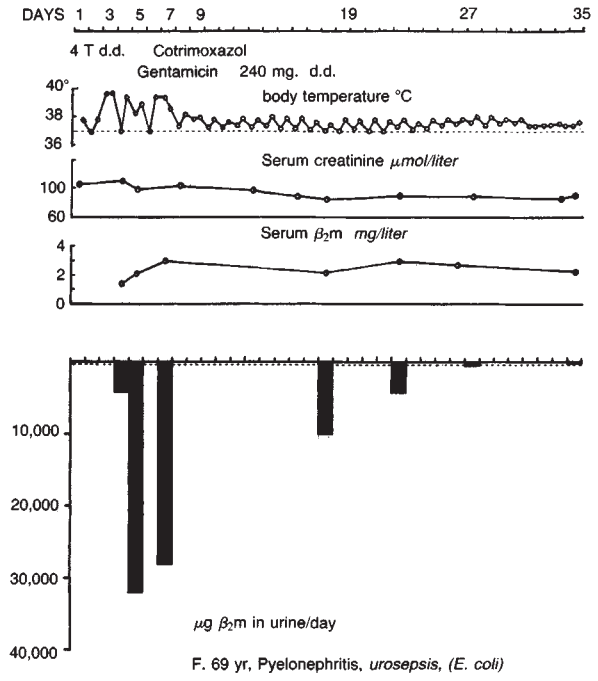

Fig. 5. *β_2-microglobulin in serum and urine in a 69-year-old female with pyelonephritis and urosepsis, demonstrating the significantly increased β_2-microglobulin excretion and its return to normal with appropriate therapy.* From [3].

icant changes of serum β_2M as a useful marker of compromised renal graft function with discrimanatory capacity and predictive value for diagnosis of graft rejection [55] or as an adjunct to, but not a replacement for, classical methods for detecting acute rejection [52].

In the interpretation of serum and urinary β_2M in kidney transplantation, a decrease of β_2M will reflect improvement of glomerular and tubular function (tubular uptake and metabolism). An increase of serum and urinary β_2M may be caused by intercurrent multiple pathologic processes like the following:

- renal tubular ischemia: renal artery stenosis, cardiovasculair insufficiency
- infection: pyelonephritis, cytomegalovirus
- post-renal obstruction
- nephrotoxic agents: cyclosporine, aminoglycoside

β_2-microglobulin and hemodialysis

Fibrils with the properties of amyloid have been isolated from patients with bone and joint involvement complicating chronic dialysis. Further analysis revealed β_2M as a major constituent of the fibrils [56, 57]. This signifies that β_2M is another example of a low molecular weight, serum protein with a potential β-pleated sheet structure, that may adopt the fibrillar configuration of amyloid in certain pathologic states. Two hundred and one patients on long-term hemodialysis had a significantly higher serum β_2M concentration than normal subjects (41.6 ± 9.6 vs. 1.2 ± 0.6 mg per liter; $P < 0.001$) [58], and it increased slightly with the duration of hemodialysis. In patients on long-term hemodialysis a new form of amyloidosis of bone is being recognized with the formation of tumoral masses sometimes presenting as pathologic fractures. The carpal tunnel syndrome has been observed increasingly in patients undergoing long-term hemodialysis treatment, the cause being amyloid deposition. A relationship has been suggested between the high serum concentration of β_2M in hemodialysis patients and the potential development of amyloidosis, carpal tunnel syndrome and of amyloid osteo-arthropathy [59], the latter being found after an average of 13 years (range 7 to 16 years) of hemodialysis.

The prevalence of amyloid osteo-arthropathy increased with time, but cases were restricted to those patients on hemodialysis with cuprophane membranes. Serum β_2M levels were higher in cuprophane than in polyacrylonitrile dialyzed patients. Hauglustaine et al [60] reported values of 50.7 ± 3.9 mg/liter and 44.5 ± 2.7 mg/liter (mean ± SEM), respectively for the two groups.

Krediet (personal communication) observed in 19, continuous ambulatory peritoneal dialysis (CAPD) patients, a serum β_2M of 35.0 mg/liter (SEM 2.5 mg/liter), which is in accordance with a higher permeability of the peritoneal membrane for low molecular weight (LMW) molecules like β_2M.

These values are significantly lower than those of hemodialysis patients mentioned above. β_2M amyloidosis has not yet been observed in CAPD patients.

Conclusion

By its unique property of being almost exclusively filtered by the glomerulus and most efficiently (99.9% of the amount filtered) reabsorbed by the cells of the proximal tubules under diverse physiologic conditions, renal β_2M excretion has proven to be a very sensitive method in diagnosing proximal tubular disorders. Many of these have been extensively investigated and are discussed in the present review. Others require more detailed research, such as the increased urinary β_2M excretion in eclampsia [3], in renal ischemia, shock and acute tubular necrosis. Renal embolization in one observation resulted in very high renal β_2M excretion [3].

Renal β_2M excretion has been successfully employed in epidemiological surveys in regions with endemic Balkan nephropathy and in discovering chronic intoxication in areas polluted by cadmium or other heavy metals.

And finally, urinary β_2M has been shown to be the most reliable test for discriminating between upper and lower urinary tract infections. It is most useful in evaluating the results of therapy and in detecting recurrences in acute pyelonephritis by means of serial determinations.

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