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Torffvit, Ole; Kalani, Majid; Apelqvist, Jan; Eliasson, Björn; Eriksson, Jan W; Brismar, Kerstin; Jörneskog, Gun

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Research Article

Increased Urine IgM and IgG2 Levels, Indicating Decreased Glomerular Size Selectivity, Are Not Affected by Dalteparin Therapy in Patients with Type 2 Diabetes

Ole Torffvit,1,2 Majid Kalani,3 Jan Apelqvist,4 Björn Eliasson,5 Jan W. Eriksson,6 Kerstin Brismar,7 and Gun Jörneskog8

1 Department of Nephrology, Institution of Clinical Sciences, Lund University Hospital, 22185 Lund, Sweden
2 Primary Care Unit, Capio-Citykliniken, Björkhemsvägen 15C, 29154 Kristianstad, Sweden
3 Department of Cardiology, Danderyd Hospital, 18288 Stockholm, Sweden
4 Department of Endocrinology, Malmö University Hospital, 20502 Malmö, Sweden
5 Diabetes Centrum, Sahlgrenska University Hospital, 41345 Göteborg, Sweden
6 Department of Molecular and Clinical Medicine, Sahlgrenska University Hospital, 41345 Göteborg, Sweden
7 Department of Endocrinology, Karolinska University Hospital, 17164 Solna, Sweden
8 Department of Clinical Sciences, Danderyd Hospital, Karolinska Institutet, 18288 Stockholm, Sweden

Correspondence should be addressed to Ole Torffvit, ole.torffvit@med.lu.se

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Fifty-four type 2 diabetic patients with neuroischemic foot ulcers were randomised to treatment with 5000 IU of dalteparin, \( n = 28 \), or physiological saline, \( n = 26 \), once daily until ulcer healing or for a maximum of 6 months. Thirty-three patients had normo-, 15 micro-, and 6 macroalbuminuria. The urinary levels of IgM and IgG2 were elevated in 47 and 50 patients, respectively. Elevated urinary levels of IgM and IgG2 indicate decreased glomerular size selectivity. Urine IgM levels were associated with IGF-1/IGFBP-1 and IGFBP-1 levels. Dalteparin treatment increased urinary levels of glycosaminoglycans (\( P < 0.001 \)) and serum IGFBP-1 (\( P < 0.05 \)) while no significant effects were seen in any of the other studied parameters. In conclusion, dalteparin therapy in patients with type 2 diabetes had no effects on urinary levels of albumin, IgM, or IgG2 despite significantly increased glycosaminoglycans in urine. Elevated urinary levels of IgM and IgG2 might be more sensitive markers of renal disease than albuminuria in patients with type 2 diabetes and antihypertensive therapy.

1. Introduction

Albuminuria is a marker of diabetic nephropathy and a strong predictor of widespread vascular damage [1]. The Steno hypothesis held that genetically based disturbances in the production or sulphation of heparan sulphate (HS) lead to a reduction of sulphated and negatively charged HS glycosaminoglycan (GAG) side chains. Negatively charged HS GAG side chains are normally found in the extracellular matrix and vascular basement membranes. High blood glucose levels lead to lower activity of the enzymes involved in GAG metabolism and sulphation of HS [2]. A reduction of negatively charged HS GAG may induce an increased transvascular permeability of negatively charged plasma proteins, which promotes vascular and glomerular changes [1, 3–5]. Positive effects of heparin on diabetic nephropathy have been shown in experimental studies [6–8]. In humans with diabetes, several studies have shown a reduction of urinary albumin excretion during treatment with unfractioned heparin, low-molecular-weight heparins (LMWH), or oral treatment with sulodexide, suggesting that these compounds can improve GAG metabolism and sulphation of HS [3]. Thus, in patients with type 1 diabetes, treatment with unfractionated heparin, sulodexide or LMWH decreased the albumin excretion rate [9, 10], whereas in type 2 diabetes, the effect on albuminuria seems less consistent [3]. In a study by
Nielsen et al., three weeks of daily injections of the LMWH tinzaparin had no effect on albuminuria in patients with type 2 diabetes [11]. We have earlier reported an improved outcome of chronic neuroischemic foot ulcers in patients with diabetes during long-term treatment with dalteparin [12]. The beneficial effects of dalteparin on ulcer outcome involved an inhibitory effect on thrombin generation and improved haemostatic and microvascular functions [13]. The described effects of dalteparin may be beneficial not only for outcome of neuroischemic diabetic foot ulcers but also for other complications, such as diabetic nephropathy. Thus, the aim of this ancillary study was to investigate the effect of treatment with the LMWH dalteparin on proteinuria in patients with diabetes and severe vascular complications. The selectivity of the glomerular filter was studied by analyzing the urinary excretion of molecules of different size and charges [14–17], that is, IgM was analysed for determination of the size, and IgG2 and IgG4 for determination of the neutral and negative charges of the glomerular filter, respectively. The glomerular mesangial matrix turnover was assessed by measuring the urinary excretion of cytokine transforming growth factor beta 1 (TGFβ1) [18]. Furthermore, we analyzed insulin-like growth factor 1 (IGF-1) and IGF-binding protein 1 (IGFBP-1) since the IGFBP-1 [19] and IGF1 have been shown to be associated with diabetes nephropathy independent of the degree of albumin [20]. It has been speculated that low IGF-1 activity may induce apoptosis or loss of podocytes and thus lead to glomerulosclerosis [21].

2. Subjects and Methods

2.1. Subjects. Of the previously described 87 diabetic patients [12] with peripheral arterial occlusive disease (PAOD) and chronic foot ulcer, 54 type 2 diabetic patients who completed the urine collections were included in the present study. All patients were treated with 75 mg aspirin once daily since at least four weeks before randomization and throughout the study period.

2.2. Methods. Prospective, double-blind, and placebo-controlled multicenter study to evaluate the effects of dalteparin (Fragmin, Pfizer) primarily on healing of neuroischemic foot ulcers [12] and secondarily on haemostatic and microvascular functions [13], and renal excretion of proteins. The patients were randomized to treatment with 0.2 mL daily subcutaneous injections of dalteparin (25000 U/mL) or physiological saline until ulcer healing or for a maximum of six months.

Timed urine collections from three consecutive nights before and at the end of treatment were stored at −20°C and analyzed at the Renal Laboratory, Lund. Microalbuminuria was defined as a mean value of the urine collections of 20 to 200 μg/min or u-albumin/creatinine ratio of 3–30 mg/mmol. An excretion below these levels was defined as normo- and an excretion above as macroalbuminuria. Urine albumin [22], total GAG [23], IgM [24], IgG2, and IgG4 [25] were analyzed as previously described. Biologically active TGFβ1 was analyzed with a commercially available assay (Emax Immunoassay System, Promega Corp., Madison, WI, USA). U-creatinine was analyzed with an enzymatic method (EKTACHEM, Clinical Chemistry Slide, Johnson & Johnson Clinical Diagnostics, Rochester, NY, USA). HbA1c was analyzed by an immunoturbidimetric method (UNIMATE 3 HbA1c, Roche Diagnostics). HsCRP and S-AA were measured using particle-enhanced immunonephelometric methods (BN, Dade Behring). IGF-1 [26] and IGFBP-1 [27] were determined in serum by radioimmunoassays (RIAs).

2.3. Statistical Methods. Data are shown as mean and SD and skewed variables as median (minimum and maximum values). For differences within subjects we used Friedman’s test, with Wilcoxon signed-rank test as post hoc test. The chi-square test was used to compare differences in the distribution of categorical variables. For testing of differences between subject groups, the Mann-Whitney U test was used. P values below 0.05 were considered significant (2-tailed). The statistical program SPSS was used.

2.4. Ethical Considerations. The study protocol was approved by the local ethics committee of each centre and the Swedish Medical Products Agency. Written informed consent was obtained from all patients.

3. Results

3.1. Patient Characteristics. Fifty-four patients with type 2 diabetes were able to leave timed urine collections from three consecutive nights before and at the end of treatment period. All patients had PAOD, peripheral neuropathy, and chronic foot ulcers. Seven patients in the dalteparin and 10 in the placebo group had suffered from myocardial infarction, and two patients in the placebo group had undergone leg amputation. Except for more ex-smokers in the placebo group, the baseline patient characteristics were not different between the two groups (Table 1). Levels of HbA1c at baseline (Table 1) and at the end of treatment period (dalteparin: 7.0 (4.9–10.8)%; placebo: 6.3 (4.6–8.7)%) were not significantly different between the groups. Ten patients in the dalteparin group and 11 in the placebo group had micro- or macroalbuminuria (Table 1). Thirty-six patients, including 23 patients with normoalbuminuria, were on antihypertensive treatment (Table 1).

3.2. Treatment Period. The treatment period with dalteparin was not significantly different from the treatment period in the placebo group. It lasted for median 26 and range 8 to 26 weeks.

3.3. Renal Parameters. At baseline, 33 patients had normo-, 15 micro-, and 6 macroalbuminuria. Thirty-six patients, including 23 patients with normoalbuminuria, were on antihypertensive treatment (Table 1). Ten patients in the dalteparin group and 11 in the placebo group had micro- or macroalbuminuria (Table 1). Forty-seven patients showed elevated urinary levels of IgM (Figure 1), while 50 patients had elevated urinary levels of IgG2, both indicating decreased
Table 1: Baseline characteristics of 54 patients randomized to dalteparin or placebo.

| Characteristic                                | All (N=54) | Dalteparin (N=28) | Placebo (N=26) |
|-----------------------------------------------|------------|-------------------|----------------|
| Age (years)                                   | 75 (54–90) | 70 (57–86)        | 75 (54–90)     |
| Gender (male/female)                          | 37/17      | 17/11             | 20/6           |
| Smoker/ex-smoker/nonsmoker (n)                | 9/14/31    | 4/3/21*           | 5/11/10        |
| HbA1c (%)                                     | 6.7 (5.0–11.0) | 6.9 (5.1–11) | 6.9 (5.0–9.6) |
| Diabetes duration (years)                     | 17 ± 9     | 17 ± 10           | 16 ± 8         |
| Tablets/insulin/tablets + insulin/diet (n)    | 10/31/8/5  | 4/18/3/3          | 6/13/5/2       |
| Antihypertensive treatment (n) (ACE/β/Ca/diuretic/other) | 10/13/5/22/4 | 5/6/3/14/0 | 5/7/2/8/4     |
| Systolic blood pressure (mmHg)                | 158 ± 22   | 160 ± 22          | 155 ± 22       |
| Diastolic blood pressure (mmHg)               | 80 ± 11    | 78 ± 9            | 82 ± 12        |
| P-Creatinine (μmol/L)                         | 83 (53–160)| 83 (57–130)       | 84 (53–160)    |
| GFR (mL/min)                                  | 74 (17–218)|(n = 22)           | 80 (17–218)    |
| Albuminuria (normo/micro/macro):              |            |                   |                |
| Baseline (n)                                  | 33/15/6    | 18/8/2            | 15/7/4         |
| At endpoint (n)                               | 35/12/7    | 18/7/3            | 17/5/4         |

* P < 0.05 versus placebo. Data are given as mean ± SD, or as median and minimum-maximum values. GFR: glomerular filtration rate; creatinine clearance.

3.4. Comparisons with Data from Control Subjects. In comparison with control subjects [28], the urinary levels of IgG2 were higher in the patients with micro- or macroalbuminuria while normal in those with normoalbuminuria. Levels of IgG4 were normal, while IgG2/IgG4 ratios, and IgM and TGFβ1-values [18] were increased irrespective of the level of albuminuria (for reference values, see Table 2).

3.5. Inflammatory Parameters, IGF-1 and IGFBP-1. The levels of hsCRP, SAA, S-IGF-1, and S-IGFBP-1 were similar in the dalteparin and placebo groups at baseline and during the treatment period (data not shown), except for S-IGFBP-1 which increased in patients with micro-macroalbuminuria in comparison with placebo-treated patients (Tables 3 and 4). No associations were found with any of the urinary parameters or HbA1c levels. S-IGF-1 was negatively associated with systolic BP at entry (r = −0.304, P = 0.048, n = 43). SAA and hsCRP were negatively associated with systolic BP at endpoint (r = −0.294, P = 0.038, n = 50 and r = −0.292,
Table 2: Baseline values in patients grouped with normo- or micro- and macroalbuminuria.

|                        | Normoalbuminuria | Micro- and macroalbuminuria |
|------------------------|------------------|-----------------------------|
| Age (years)            | N = 33           | N = 21                      |
|                        | 74 (54–90)       | 75 (61–86)                  |
| Diabetes duration (years) | 15 ± 9     | 19 ± 8                      |
| Gender (male/female)   | 21/12            | 16/5                        |
| Systolic blood pressure (mmHg) | 150 (115–210) | 160 (135–215)               |
| Diastolic blood pressure (mmHg) | 80 (60–100) | 85 (60–105)                 |
| S-HbA1c (%)            | 6.5 (5.0–9.9)    | 6.9 (5.1–11.0)              |
| S-Creatinine (μmol/L) | 81 (53–160)      | 85 (65–128)                 |
| S-Hs CRP (mg/L)        | 9.4 (0.9–118)    | 2.7 (0.3–78.2)              |
| S-AA (mg/L)            | 5.5 (1.2–415)    | 5.1 (1.7–127)               |
| S-IGF-1 (μg/L)         | 134 (47–384)     | 115 (49–269)                |
| S-IGFBP-1 (μg/L)       | 41 (15–310)      | 60 (8–313)                  |
| U-Glycosaminoglycan (mg/mmol) | 2.7 (0–8.7) | 2.6 (0–11.1)               |
| U-IgG2 (mg/mmol)       | 0.18 (0–8.1)     | 0.85 (0–99)                 |
| U-IgG4 (mg/mmol)       | 0.06 (0–7.7)     | 0.27 (0–28.7)               |
| U-IgG2/IgG4            | 3.1 (0.04–31.0)  | 3.3 (0.76–10.5)             |
| U-IgM (mg/mmol)        | 0.02 (0–0.06)    | 0.03 (0–0.13)               |
| U-TGFβ1 (mg/mmol)      | 3.2 (1.1–379)    | 4.5 (1.4–16.5)              |

*P < 0.05 versus normoalbuminuria. Data are given as median and range (min-max). Urine data are the ratio between urine protein and urine creatinine.

Table 3: Diabetic patients with normoalbuminuria: effects of treatment on urinary indices.

|                        | Dalteparin | Placebo |
|------------------------|------------|---------|
|                        | Baseline   | At endpoint |
|                        | n = 18     | n = 18   |
| U-Albumin (mg/mmol)    | 0.81 (0.07–2.39) | 0.77 (0.06–4.97) |
| U-IgG2 (mg/mmol)       | 0.19 (0–8.14)   | 0.14 (0–7.79)   |
| U-IgG4 (mg/mmol)       | 0.05 (0–7.68)    | 0.04 (0–1.08)    |
| U-IgG2/IgG4            | 3.49 (0.04–31)   | 2.20 (0.37–44.59) |
| U-GAG (mg/mmol)        | 2.43 (0.86–8.65) | 2.85 (1.32–8)   |
| U-IgM (mg/mmol)        | 0.02 (0–0.06)    | 0.02 (0–0.05)    |
| TGF-β1 (mg/mmol)       | 3.2 (1.1–379)    | 5.17 (1.47–21.3) |
| GFR (mL/min)           | 70 (34–190)     | 65 (33–163)     |
| IGFBP-1 (μg/L)         | 42 (21–310)     | 49 (27–315)     |

Data are given as the median (with minimum and maximum values in parentheses) of the ratio between urinary concentrations of substance and u-creatinine. *P < 0.05 versus placebo; #P < 0.05 versus baseline. GFR: glomerular filtration rate: creatinine clearance.

4. Discussion

The results of the present study show that six months of treatment with the LMWH dalteparin had no effect on glomerular function, inflammatory parameters, or urinary levels of proteins despite an increased urinary excretion of GAG. Our results extend the findings of an earlier study showing that three weeks of LMWH treatment had no effect on albuminuria in patients with type 2 diabetes [11]. These findings are in contrast to the effect seen in type 1 diabetic patients showing a reduced albuminuria during one-to-three month treatment with either unfractionated heparin or LMWH [9, 10]. The reason for this discrepancy in effects of heparins on urinary excretion of proteins between patients with type 1 and type 2 diabetes is unclear and cannot be explained by the present study. However, the structure of the heparin...
molecule might be of importance since mixed compositions of sulphated GAG and heparan sulphate, for example, dana-molecule might be of importance since mixed compositions

Data are given as the median (with minimum and maximum values in parentheses) of the ratio between urinary concentrations of substance and u-creatinine.

| Table 4: Diabetic patients with micro- or macroalbuminuria: effects of treatment on urinary indices. |
|--------------------------------------------------|--------------------------------------------------|
| Dalteparin | Placebo |
| Baseline | At end point | Baseline | At end point |
| **U Albmin** (mg/mmol) | 8.5 (0.9–435) | 11.3 (1.5–311) | 23.2 (2.1–187) | 7.9 (0.9–273) |
| **U IgG2** (mg/mmol) | 0.46 (0–20.7) | 0.83 (0.13–35.1) | 2.99 (0.02–99.4) | 2.60 (0–70.0) |
| **U IgG4** (mg/mmol) | 0.21 (0–3.94) | 1.02 (0.04–7.16) | 0.50 (0.03–28.7) | 0.15 (0–53.7) |
| **U IgG2/u IgG4** | 1.88 (1–10.5) | 3.05 (0.33–9.16) | 5.57 (0.8–9.7) | 4.47 (0.35–24.1) |
| **U GAG** (mg/mmol) | 2.31 (0–4.52) | 3.97 (1.25–6.1) | 2.70 (0–11.1) | 2.49 (0–5.19) |
| **U IgM** (mg/mmol) | 0.03 (0–0.05) | 0.03 (0.01–0.12) | 0.03 (0–0.13) | 0.02 (0–0.14) |
| **TGF b1** (mg/mmol) | 4.44 (1.4–15.9) | 4.09 (2.14–14.12) | 4.5 (1.8–16.5) | 3.5 (1.19–22.44) |
| **GFR** (mL/min) | 74 (36–140) | 76 (18–208) | 71 (38–107) | 67 (34–113) |
| **IGFBP-1** (μg/L) | 66 (8–313) | 105 (23–219) | 60 (20–130) | 46 (10–161) |

Data are given as the median (with minimum and maximum values in parentheses) of the ratio between urinary concentrations of substance and u-creatinine.

*P < 0.05 versus placebo; **P < 0.01 versus baseline. GFR: glomerular filtration rate; creatinine clearance.
with local effect in the kidney. In the present study in patients with vascular disease we found increased excretion of IgM, and thus these patients may be at increased risk. We furthermore found a positive association between IGFBP-1 and excretion of IgM indicating that high IGFBP-1 may be associated with glomerular damage. Thus, we were able to confirm decreased levels of IGF-1 and increased levels of IGFBP-1 in type 2 diabetes patients with nephropathy [20]. Furthermore, IGFBP-1 increased to significantly higher levels in patients treated with dalteparin than in placebo-treated ones. The reason for these increased levels is not known but may be due to reduced proteolysis of IGFBP-1. In line with a study by Sharma et al. [18], the present study showed increased urinary levels of TGFβ1 in patients with type 2 diabetes. However, the levels of TGFβ1 were also unaffected by dalteparin treatment.

In conclusion, the present study showed no effects of dalteparin on the glomerular filter despite increased S-IGFBP-1 levels and urinary levels of GAG. Thus, the study indicates that proteinuria in type 2 diabetic patients may be caused by an alteration of the size-selective properties of the glomerular capillary wall. IgM and IgG2 seem to be better markers than albuminuria for severe vascular disease.

**Abbreviations**

- IgG: Immunoglobulin G
- TGFβ1: Transforming growth factor β1
- IGF-1: Insulin-like growth factor 1
- IGFBP-1: Insulin-like growth factor binding protein 1.

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**References**

[1] T. Deckert, B. Feldt-Rasmussen, K. Borch-Johnsen, T. Jensen, and A. Kofoed-Enevoldsen, "Albuminuria reflects widespread vascular damage. The steno hypothesis," *Diabetologia*, vol. 32, no. 4, pp. 219–226, 1989.

[2] A. Kofoed-Enevoldsen, D. Noonan, and T. Deckert, "Diabetes mellitus induced inhibition of glucosaminyl N-deacetylase: effect of short-term blood glucose control in diabetic rats," *Diabetologia*, vol. 36, no. 4, pp. 310–315, 1993.

[3] G. Gambaro and F. J. Van Der Woude, "Glycosaminoglycans: use in treatment of diabetic nephropathy," *Journal of the American Society of Nephrology*, vol. 11, pp. 359–368, 2000.

[4] J. T. Tamsma, J. Van Den Born, J. A. Bruin et al., "Expression of glomerular extracellular matrix components in human diabetic nephropathy: decrease of heparan sulphate in the glomerular basement membrane," *Diabetologia*, vol. 37, no. 3, pp. 313–320, 1994.

[5] N. P. Goode, M. Shires, D. M. Crelin, S. R. Aparicio, and A. M. Davison, "Alterations of glomerular basement membrane charge and structure in diabetic nephropathy," *Diabetologia*, vol. 38, no. 12, pp. 1455–1465, 1995.

[6] P. S. Oturai, R. Rasch, E. Hasselager et al., "Effects of heparin and aminoguanidine on glomerular basement membrane thickening in diabetic rats," *Acta Pathologica, Microbiologica et Immunologica Scandinavica*, vol. 104, no. 4, pp. 259–264, 1996.

[7] S. M. Marshall, K. W. Hansen, R. Østerby, J. Frystyk, H. Ørskov, and A. Flyvbjerg, "Effects of heparin on renal morphology and albuminuria in experimental diabetes," *American Journal of Physiology*, vol. 271, no. 2, pp. E326–E332, 1996.

[8] I. Ichikawa, Y. Yoshida, A. Fogo, M. L. Purkerson, and S. Klahr, "Effect of heparin on the glomerular structure and function of remnant nephrons," *Kidney International*, vol. 34, no. 5, pp. 638–644, 1988.

[9] B. Myrup, P. M. Hansen, T. Jensen et al., "Effect of low-dose heparin on urinary albumin excretion in insulin-dependent diabetes mellitus," *The Lancet*, vol. 345, no. 8947, pp. 421–422, 1995.

[10] J. T. Tamsma, F. J. Van Der Woude, and H. H. P. J. Lemkes, "Effect of sulphated glycosaminoglycans on albuminuria in patients with overt diabetic (type 1) nephropathy," *Nephrology Dialysis Transplantation*, vol. 11, no. 1, pp. 182–185, 1996.

[11] S. Nielsen, A. Schmitz, T. Bacher, M. Rehling, J. Ingelslev, and C. E. Mogensen, "Transcapillary escape rate and albuminuria in type II diabetes. Effects of short-term treatment with low-molecular weight heparin," *Diabetologia*, vol. 42, no. 1, pp. 60–67, 1999.

[12] M. Kalani, J. Apelqvist, M. Blombäck et al., "Effect of dalteparin on healing of chronic fott ulcers in diabetic patients with peripheral arterial occlusive disease: a prospective, randomised, double-blind and placebo-controlled study," *Diabetes Care*, vol. 26, no. 9, pp. 2575–2580, 2003.

[13] M. Kalani, A. Silveira, J. Apelqvist et al., "Beneficial effects of dalteparin on haemostatic function and local tissue oxygenation in patients with diabetes, severe vascular disease and foot ulcers," *Thrombosis Research*, vol. 120, no. 5, pp. 653–661, 2007.

[14] Y. Chiba, N. Tani, M. Yamazaki, H. Nakamura, S. Ito, and A. Shibata, "Glomerular charge selectivity in non-insulin-dependent diabetes mellitus," *Journal of Diabetes and Its Complications*, vol. 5, no. 2–3, pp. 135–137, 1991.

[15] S. Morano, P. Pietravelle, M. G. De Rossi et al., "A charge selectivity impairment in protein permselectivity is present in type 2 diabetes," *Acta Diabetologica*, vol. 30, no. 3, pp. 138–142, 1993.

[16] M. A. Gall, A. Kofoed-Enevoldsen, F. S. Nielsen, and H. H. Parving, "Glomerular size- and charge selectivity in type 2 (non-insulin-dependent) diabetic patients with diabetic nephropathy," *Diabetologia*, vol. 37, no. 2, pp. 195–201, 1994.

[17] K. Yoshioka, S. Tanaka, M. Imanishi et al., "Glomerular charge and size selectivity assessed by changes in salt intake in type 2 diabetic patients," *Diabetes Care*, vol. 21, no. 4, pp. 482–486, 1998.
[18] K. Sharma, F. Ziyadeh, B. Alzahabi et al., “Increased renal production of transforming growth factor-β1 in patients with type II diabetes,” *Diabetes/Metabolism Reviews*, vol. 46, no. 5, pp. 854–859, 1997.

[19] R. Stephens, P. McElduff, A. Heal et al., “Polymorphisms in IGF-binding protein 1 are associated with impaired renal function in type 2 diabetes,” *Diabetes/Metabolism Reviews*, vol. 54, no. 12, pp. 3547–3553, 2005.

[20] M. Akturk, M. Arslan, A. Altinova et al., “Association of type II diabetes,” *Diabetes/Metabolism Reviews*, vol. 34, no. 4, pp. 975–981, 2011.

[21] O. Tor, F. van der Woude, P. Geelhoed-Duijvestijn et al., “Danaparoid sodium lowers proteinuria in diabetic nephropathy,” *American Journal of Kidney Diseases*, vol. 58, no. 5, pp. 729–736, 2011.

[22] J. Dawes, C. Prowse, and D. S. Pepper, “Absorption of heparin, LMW heparin and SP54 after subcutaneous injection, assessed by competitive binding assay,” *Thrombosis Research*, vol. 44, no. 5, pp. 683–693, 1986.

[23] K. Chaudhary, G. Phadke, R. Nivastala, C. Weidmeyser, S. McFarlane, and A. Whaley-Connell, “The emerging role of biomarkers in diabetic and hypertensive chronic kidney disease,” *Current Diabetes Reports*, vol. 10, no. 1, pp. 37–42, 2010.

[24] F. Nauta, W. van Oeveren, W. Boertien et al., “Glomerular and tubular damage markers are elevated in patients with diabetes,” *Diabetes Care*, vol. 34, no. 4, pp. 975–981, 2011.

[25] W.-J. Fu, S.-L. Xiong, Y.-G. Fang et al., “Urinary tubular biomarkers in short-term type 2 diabetes mellitus patients: a cross-sectional study,” *Endocrine Journal*, vol. 41, no. 1, pp. 82–88, 2012.

[26] G. Tramonti and Y. S. Kanwar, “Tubular biomarkers to assess progression of diabetic nephropathy,” *Kidney International*, vol. 79, no. 10, pp. 1042–1044, 2011.

[27] S. Nielsen, S. Andersen, D. Zdunek, G. Hess, H.-H. Parving, and P. Rossing, “Tubular markers do not predict the decline in glomerular filtration rate in type 1 diabetic patients with overt nephropathy,” *Kidney International*, vol. 79, no. 10, pp. 1113–1118, 2011.

[28] O. Bakoush, O. Torffvit, B. Rippe, and J. Tencer, “High proteinuria selectivity index based upon IgM is a strong predictor of poor renal survival in glomerular diseases,” *Nephrology Dialysis Transplantation*, vol. 16, no. 7, pp. 1357–1363, 2001.