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Change and significance of serum inflammatory factors, vWf, VEGF and adhesion molecules in patients with diabetic nephropathy

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ABSTRACT

Objective: To investigate the change of serum inflammatory factors, von Willebrand factor (vWf), vascular endothelial growth factor (VEGF) and adhesion molecule levels in diabetic nephropathy patients (DN), and their significance in the detection of diabetic nephropathy. **Methods:** According to urinary albumin excretion rate (UAER), a total of 158 cases of diabetic patients were divided into T2DM group (Simple diabetes group, *n*=52) and DN group (diabetic nephropathy group, *n*=106), group DN were divided into microalbuminuria group (*n*=54) and macroalbuminuria group (*n*=52); At the same time, 50 healthy subjects were selected as control group, the levels of serum inflammatory factors (hs-CRP, IL-6 and TNF-α), vWf, VEGF and adhesion molecules (sVCAM-1, sICAM-1 and E- selectin) in these four groups were compared. **Results:** The levels of hs-CRP, IL-6, vWf, VEGF, TNF-α, sVCAM-1, sICAM-1 and E- selectin in the T2DM group and DN group were significantly higher than those in the control group, and DN group was significantly higher than that in T2DM group, the difference was statistically significant; In the DN group, compared with microalbuminuria group, the levels of hs-CRP, IL-6, vWf, VEGF, TNF-α, sVCAM-1, sICAM-1 and E- selectin in the macroalbuminuria group were significantly increased, the difference was statistically significant. **Conclusion:** Serum inflammatory factors, vWf, VEGF and adhesion molecules may play important roles in the course of the genesis and development of DN. It has great value to the assessment of this disease.

1. Introduction

Diabetic nephropathy (DN) was principle microangiopathy of type 2 diabetes mellitus and was a main reason of kidney diasease at terminal stage and DM patients′ death[1]. It has been proved that inflammatory factor and vascular endothelian injury played critical role in genesis and development of DN[2,3]. Von Willebrand factor (vWf) and vascular endothelial growth factor (VEGF) were important markers of vascular endothelian injury, which could reflect vascular injury and hypercoagulability condition. Research pointed out that adhension molecules level could reflect inflammation degree and closely related to insulin resistance, most of researchers payed attention on its relationship with DN[4,5]. This research was aimed to explore change and significance of serum inflammatory factors, von Willebrand factor (vWf), vascular endothelial growth factor (VEGF) and adhesion molecule levels in diabetic nephropathy patients (DN).

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2 Research objects and method

2.1. Research objects

A total of 158 cases of T2DM patients who were admitted in endocrinology department of our hospital from Februry 2015 to June 2017, all patients were conformed to T2DM and DN related diagnostic standard[6,7]. According to urinary albumin excretion rate (UAER), patients were divided into pure diabetes group (T2DM group, UAER<20 μg/min, *n*=52) and diabetic nephropathy group (DN group, UAER 20 μg/min, n=106), DN group was divided into microalbuminuria group (20 μg/min UAER <200 μg/min, *n*=54) and macroalbuminuria group (UAER 200 μg/min, *n*=52).

In T2DM group 34 males, 18 females, aged from 35-75 years old; microalbuminuria group 33 males, 21 females, aged from 34-76 years old; macroalbuminuria group 35 males, 17 females, aged from 34-75 years old; At the same time, 50 healthy subjects were selected as control group, 35 males, 15 females, aged from 34-76 years old. Gender and age of four groups subject were no statistical significnat difference (P>0.05), it was comparabe. Exclusion: (1) patinets with severe heart and liver dysfunction, tumor and acute and chronic infective disease; (2) combined with other disease that affected UAER level (such as urinary system infection); (3) combined with hypertension; (4) in recent, sufferd from diabetic ketoacidosis and hyperosmotic coma; (5) accepted angiotension converting enzyme inhibitor, angiotension II receptor antogonist, antiplatelet and anticoagulation drugs in recent. Ths research was confromed and approved by ethic committee of hospital.

2.2. Research method

Extracted fasting periphery venous blood of all subjects after admission or examination, after centrifudge, obtained serum and stocked at -80 ℃ freezer for detection. Detection indexes including serum inflammatory factors, vWf, VEGF and adhesion molecules. Inflammatory factor including hypersensitive C-reaction protein (hs-CRP), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α); adhnsion molecules contained vascular cell adhnsion molecule (VCAM-1), intercelular adhnsion molecule (ICAM-1) and E-selectin. hs-CRP level was detected by latex-enhanced immunoturbidimetric assay (Kits was purchased from Shanghai Xinyu biotechnoloy Co.,Ltd.), IL-6, TNF-α, vWf, VEGF, VCAM-1, ICAM-1 and E-selectin level were detected by double antibody sandwich ELISA, its coresponding ELISA kits were provided by Shanghai Meilian biotechnoloy Co.,Ltd, operation was stricted with kits introduction.

2.3. Statistcal analysis

Statistical Software SPSS17.0 was used for all row data processing and analyzing, inflammatory factors, vWf, VEGF and adhesion molecule level was conformed to normal distribution and represented by (Mean ± SD), -way analysis of variance was adopted to multiple interlock comparison, SNK-q-test was applied to comparison of intra-group two inter-block, P<0.05 indicated the difference was statistical significant.

3. Results

3.1. Comparison of serum inflammatory factor level in four groups

Detection results of serum inflammatory factor level in four groups were shown in Table 1. hs-CRP, IL-6 and TNF-α level in DN group were respectively (7.52±3.45) mg/L, (166.98±60.06) ng/L and (21.96±8.87) ng/L. Compared with control group, hs-CRP, IL-6 and TNF- level in T2DM and DN group were increased dramatically (P<0.05), and DN group was significantly higher than T2DM group, the difference was significantly statistical (P<0.05); these three level in macroalbuminuria group were higher than microalbuminuria group, there was significant difference (P<0.05).

3.2 Comparison of vWf, VEGF level in four groups

Decteion results of serum vWf, VEGF level in four groups was shown in Table 2. vWf, VEGF level in DN group were respectively (958.61±125.65) U/L and (175.34±22.96) ng/L. Compared with control group, vWf, VEGF level in T2DM and DN group were increased dramatically (P<0.05), and DN group was significantly

Table 1.
Comparison of serum inflammatory factor level in four groups.

Group	n	hs-CRP (mg/L)	IL-6 (ng/L)	TNF-α (ng/L)
Control group	52	1.69±0.63	94.05±17.85	8.05±2.23
T2DM group	54	3.62±1.54[a]	113.54±34.72[a]	11.06±2.76[a]
DN group	102	7.52±3.45[ab]	166.98±60.06[ab]	21.96±8.87[ab]
Microalbuminuria group	52	6.89±2.63	141.82±42.66	15.63±5.54
Macroalbuminuria group	50	8.79±3.51[c]	198.84±63.41[c]	27.16±8.35[c]

Note: compared with control group, [a]P<0.05; compared with T2DM group, [b]P<0.05; compared with microalbuminuria group, [c]P<0.05.

Table 2.
Comparison of vWf, VEGF level in four groups.

Group	n	vWf (U/L)	VEGF (ng/L)
Control group	52	179.71±35.76	109.45±21.06
T2DM group	54	491.98±60.39[a]	137.58±19.42[a]
DN group	102	958.61±125.65[ab]	175.34±22.96[ab]
Microalbuminuria group	52	844.62±61.68	167.94±22.31
Macroalbuminuria group	50	1 065.15±116.17[c]	184.69±23.08

Note: compared with control group, [a]P<0.05; compared with T2DM group, [b]P<0.05; compared with microalbuminuria group, [c]P<0.05.

Table 3.
Comparison of serum adhension molecules in four groups.

Group	*n*	sVCAM-1 (μg/L)	sICAM-1 (μg/L)	E-selectin (μg/L)
Control group	52	519.56±92.38	286.15±55.24	38.22±16.34
T2DM group	54	590.57±133.86[a]	361.25±76.16[a]	54.57±23.41[a]
DN group	102	829.62±206.47[ab]	486.34±94.47[ab]	79.05±33.45[ab]
Microalbuminuria group	52	730.68±195.29	427.01±78.94	69.52±26.35
Macroalbuminuria group	50	896.27±238.16[c]	546.27±91.23[c]	87.43±34.28[c]

Note: compared with control group,[a]P<0.05; compared with T2DM group, [b]P<0.05; compared with microalbuminuria group, [c]P<0.05.

higher than T2DM group, the difference was significantly statistical (P<0.05); compared with microalbuminuria group, vWf, VEGF level in macroalbuminuria group were enhanced obviously, there was significant difference (P<0.05).

3.3. Comparison of serum adhension molecules in four groups

sVCAM-1, sICAM-1 and E-selectin level in DN group were respetively (829.62±206.47) μg/L, (486.34±94.47) μg/L and (79.05±33.45) μg/L. sVCAM-1, sICAM-1 and E-selectin level in T2DM and DN group were higher than contol group, moreover DN group was significantly higher than T2DM group, the difference was significantly statistical (P<0.05); In DN group, sVCAM-1, sICAM-1 and E-selectin in macroalbuminuria group were higher obviously than that in microalbuminuria group, there was significant difference (P<0.05). As shown in Table 3.

4. Discussion

In recent, along with aging of population aggravating and the chage of life style, genesis of DM was in increasing trend year by year, which has been the third chronic non-infectious disease that threated severely human health[8]. DN belonged to typical microvascular complication, in our country, there was about 37.4% of patients with DN in all T2DM patients[9]. The pathogenesis of DN was still not definite, but many of researches have pointed that its genesis was related to glucose and lipid metabolism disorder, change of haemodynamics, cytokines and inheritance and other factors[10–12]. DN was latent and with serious harm, therefore, study about DN pathogenesis was important for diagnosing and preventing of DN. UAER was critical symbol that evaluated the severe degree of DN condition in clinical[13]. This research was aimed to explore change and significance of serum inflammatory factors, vWf, VEGF and adhesion molecule levels under NAER level.

Related research recently pointed out that DN was a chronic inflammatory reaction that mediated by multiple cytokines, its genesis was resulted from inflammatory stress reaction on the base of glucose metabolism and change of haemodynamics[14]. Inflammatory factors were with multiple biological activity. Under normal physiological condition, inflammatory factors could maintain kidney normal structure and function, however, under pathological state, it could aggravate change of glomerulus pathological structure and function and trigger renal damage[15]. hs-CRP, IL-6 and TNF-α were important inflammatory indexes in clinical. hs-CRP was a critical reaction protein at acute stage, its level reflected inflammatory cell level, its high-low level and microangiopathy were significant relevance[16]. IL-6 was a complex cytokine in body and an important pro-inflammation factor, with multiple biological activity that could activate B cell and T cell proliferation and differentiation, activate liver cell compose acute phase reaction protein. TNF-α was a critical transmitter in inflammatory reaction and series of pathophysiology process which could induce adhesion molecule in vascular endothelial of glomerulus and inflammatory cell expression, change hemodynamics of glomerulus and increase vascular permeability[18]. This research revealed that compared with healthy control group, serum hs-CRP, IL-6 and TNF-α in T2DM and DN group was abnormally increased, moreover DN group was enhanced more obviously, this suggested there was varying degree of inflammatory stress reaction in T2DM and DN group, results were conformed to previous report[19]. This research also found that along with UAER level increasing, inflammatory factors increases more obvious, research results revealed that inflammatory factor participated genesis and development of DN, indicated inflammatory factors level increased of T2DM patients could increase risk of DN genesis.

Many researches have demonstrated that genesis and development of DN was related to vascular endothelial injury and dysfunction[20]. Vascular endothelial dysfunction could promote sedimentation of glomerulus extracellular matrix, thereby reduce glomerulus filtration rate and induce renal damage. vWf was mainly composed by vascular endothelial cell, when vascular endothelial dysfunction, its release increased significantly, which reflected sensitively activation and damage of vascular endothelial cell. Related study demonstrated that vWf level of T2DM patients was positive relevance with insulin resistance and fibrinogen, proving vWf high expression could reflect hypercoagulation and it was critical incentive of DN genesis[21]. VRGF could promote vascular endothelial cell proliferation, formation of newborn vessels and maintain integrality of vascular endothelial function, which was considered as one of main nosogenesis of DM retinopathy[22]. This research showed that vWf and VEGF level in T2DM and DN patients were higher than control group, moreover along with disease becoming more serious, its level increased more obviously. This research revealed that there was obvious vascular endothelial injury and dysfunction in T2DM and DN patients, vWf and VEGF played critical role in genesis and

development of DN, further demonstrated vascular endothelial injury closely related to development of DN genesis.

Adhesion molecules were glycoprotein that mediated adhesion function of cellular and intercellular matrix and was important factor that of cell activation and signal transmission and other pathophysiological process. It have been demonstrated that VCAM-1, ICAM-1highly expressed in T2DM patients[23]. VCAM-1, ICAM-1 mainly mediated adhesive function between leukocyte and vascular endothelial cell, induced vascular extracellular matrix sedimentation and vascular permeability increased. E-selectin was specific index of endothelial cell activation, it did not express or lowly expressed under normal condition, its high expression could mediate adhesion and transfer of multiple leukocytes, generate and release platelet activating factor, cause microcirculation dysfunction[24]. This result found that high expression of adhesion molecule in T2DM and DN patients and along with UAER increasing, its level enhanced more obviously, the reason might be related to lipid peroxidation that induced by hyperglycemia for long time[25].

In conclusion, compared with healthy people, there was obvious inflammatory stress reaction and vascular endothelial injury, high adhesion molecule level. Under different UAER level, hs-CRP, IL-6, TNF- , vWf, VEGF, sVCAM-1, sICAM-1 and E-selectin of patients was significantly abnormal, moreover, with severity increasing, its abnormality was more obvious. It has been demonstrated that serum inflammatory factor, vWf, VEGF and adhesion molecules played roles in genesis and development of DN. Detected their levels change was with significant value for preventing of genesis and development of DN.

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