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Relationship between serum sialic acid and neuraminidase activity in rats with diabetes and renal injury

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Abstract

Introduction: Neuraminidase (NA) activity and sialic acid are demonstrated as inflammatory markers.
Objectives: This study was designed to investigate the association of serum sialic acid and neuraminidase activity in rats with diabetes, diabetic nephropathy and renal injury.
Materials and Methods: Sixty-eight male rats were included in this study and randomly assigned to nephropathy, diabetic, diabetic nephropathy, and the control groups. Nephropathy rats group underwent injection of glycerol 50% in femur muscle. Control group was injected 2.5 cc saline in their femur and they did not intake anything by their mouth after 48 hours. Diabetic rats group was injected with a single dose injection of streptozotocin (STZ) in the tail. Control group for this mentioned group was injected with 1 mL saline in their tail. Diabetic nephropathy cases were injected STZ prepared in saline solution for 4 consecutive days (after an overnight fast) and 10 controls were injected with 1 mL saline. Serum prepared from venous blood of rats, serum sialic acid and neuraminic acid were measured.
Results: Serum sialic acid and neuraminic acid in all trial groups were significantly higher than those of the control group ($P < 0.001$). The value of serum sialic acid and neuraminic acid was significantly higher in the diabetic nephropathy group compared to nephropathy group respectively ($P < 0.01$and $P < 0.05$ respectively). In addition, serum sialic acid was significantly higher in the diabetic nephropathy group compared to diabetic group ($P < 0.01$).
Conclusion: The main finding of this study is that elevated serum neuraminic acid and sialic acid were strongly related to the presence of diabetic nephropathy in our study groups. Further research is required on the prognosis of these two inflammatory markers in diabetes and nephropathy situation.

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Introduction

Diabetic nephropathy as a microvascular complication of diabetes is the main cause of increasing chronic kidney disease. According to recent studies, inflammation is considered as a key factor in promoting diabetic nephropathy. Proteinuria, reduced glomerular filtration rate, podocyte injury, mesangial matrix expansion are the main characteristics of diabetic nephropathy. Cytokines activation, inflammation, and vascular growth have an important role in matrix accumulation in diabetic nephropathy. Kashihara et al evaluated pathogenesis of this disease and revealed that renin–angiotensin–aldosterone system, oxidative stress and transforming growth factor beta (TGF-β) relatively are involved in the pathogenesis process (1). Evidence has shown that cell adhesion molecules, growth factors, chemokines and pro-inflammatory cytokines expression are elevated in diabetic nephropathy. Serum high concentration of neuraminidase (NA) and sialic acid are demonstrated as inflammatory markers in some pathological states probably regarded to the increased levels of richly sialylated acute-phase glycoproteins (2).

Sialic acid is a protein-bound carbohydrate comprised of nine-carbon backbone, which is typically found as a component of glycan chains of all cell types. Sialic acid is the name for acetylated neuraminic acids group, such

Core tip
Our experimental study showed that elevated serum neuraminic acid and sialic acid were strongly related to the presence of diabetic nephropathy.

as N-acetyl neuraminic acid, N-glycolyl neuraminic acid, and Di-acetyl neuraminic acid. Around 90% of serum sialic acid is bound to alpha and beta globulins and free sialic acid is removed from plasma by the kidneys by way of being filtered through glomeruli, but not reabsorbed by the tubules. Concentration of serum sialic acid is abnormally high in some pathological states like tissue destruction, tissue proliferation, depolymerization or inflammation, as seen in renal disease, diabetes, in a variety of central nervous system disorders, ovarian cancer, and arthritis (4).

Neuraminidase (NA) enzymes hydrolyse the terminal α2–3, α2–6, α2–8 linkages, which bind N-acetyl-neuraminic acid to N-acetyl hexosamines and N-or O-acetylated neuraminyl residues in oligosaccharides of glycolipids and glyco-proteins. There are two major classes of neuraminidase that cleave exo or endo poly-sialic acids; exo hydrolysis of α-(2→3)-, α-(2→6)-, α-(2→8) glycosidic linkage of terminal sialic acid residues, and endo hydrolysis of (2→8)-α-sialosyl linkages in oligo- or poly sialic acids. The most abundant derivative in humans is N-acetyl neuraminic acid. Recent evidence illustrates the potential role of neuraminidase in diverse metabolic pathways including glucose homeostasis. NA activity directly corresponds to the serum sialic acid level and has been proven to increase in diabetic rats (5).

Objectives

The aim of this study was to investigate the significance of serum sialic acid and neuraminidase levels in detecting diabetes, diabetic nephropathy and renal injury.

Methods and Materials

Animals

Eight-week-old Sprague-Dawley male rats (weight of 220 to 280 gram) were randomly assigned to four groups. Nephropathy (N) rats group (n=12) underwent injection of glycerol 50% in the femur muscle (10 mL/kg body weight in saline solution) (6). This group was compared with control group (n=12); which was injected with 2.5 cc saline (10 mL/kg body weight) in their femur since they did not intake anything by their mouth after 48 hours. Diabetic (D) rats group (n=12) were administered by a single dose injection of streptozotocin (STZ) in the tail (37 mg/kg body weight prepared in saline solution). Control group for this mentioned group was injected with 1 mL saline in their tail. Diabetic nephropathy (n=10) cases were injected with 37 mg/kg body weight STZ prepared in saline solution for 4 consecutive days (after an overnight fasting) and 10 controls were injected with 1 mL saline (7).

Methods

The serum samples were immediately separated from the blood of venous in tails of rats and stored at -70°C until analyzed. Serum sialic acid was measured using the colorimetric thiobarbituric acid procedure developed by Denny et al (8). NA was purchased from Sigma (St. Louis, MO). Measurement of the serum neuraminidase activity was performed by a coupled enzyme assay as described by Ziegler and Hutchinson (9). All the chemical substances, thiobarbituric acid, sodium meta-periodate, sodium sulfate, Na-arsenate and phosphotungstic acid were obtained from Merck (Darmstat, Germany) and N acetyl neuraminic acid was prepared from Fluka (Switzerland). Blood glucose was measured by glucose oxidase assay and creatinine was measured by Jaffe's method, using commercial kits (Pars Azmoon Co., Tehran, Iran) and UV-Vis spectrophotometer (Unico, USA).

Ethical issues

All experimental protocols and steps of the tests were conducted in accordance with the regulations of the Research Ethics Committee of Iranian Ethical Guidelines for the use of animals in research. Additionally, all animal experiments were in accordance with protocols approved by the United States National Institutes of Health (NIH, 1978). This study was approved and supported by Ethics Committee of Shiraz University of Medical Sciences (grant # 1186-80). This study was also extracted from master's thesis of Rita Arabsolghar in the School of Medicine at Shiraz University of Medical Sciences.

Statistical analysis

Data were analyzed by Statistical Package for Social Sciences software, version 20.0 (SPSS Inc. Chicago, IL). Data were presented as the mean ± standard deviation (SD). All variables in each group had normal distribution. The comparison of continuous variables between different groups was carried out by one-way analysis of variance (ANOVA) followed by Tukey HSD, Duncan post hoc tests, and independent *t* test. A *P* value of less than 0.05 was considered as significant.

Results

Four days after STZ injection, the serum glucose in diabetic (D) and diabetic nephropathy (DN) group had been > 300 mg/100 mL. Serum level of creatinine was >2 mg/100 mL after two days in nephropathy (N) and DN groups (Table 1). Figure 1 displays serum sialic acid measurement and demonstrates that serum sialic acid in all trial groups is significantly higher than their control group ($p< 0.001$). The value of the serum sialic acid was elevated in the diabetic nephropathy group compared to N and D groups [80.91 ± 2.35 in DN group versus 72.41 ± 2.07 in N group($P < 0.01$)] and 80.91 ± 2.35 in DN group versus 67.39 ± 1.91 in D group ($P < 0.01$), respectively. As shown in Figure 2 after nephropathies and diabetes induction, the serum neuraminidase activity was significantly elevated in each group compared to control ($P<0.001$). Serum neuraminidase activity increased considerably in the DN group compared to N (53.27 ±

Table 1. Average blood glucose and creatinine levels of diabetic and nephropathy groups

Group	Diabetic	Diabetic Nephropathy	Nephropathy
Creatinine (mg/100 mL)	-	2.04±.30	2.77±.420
Glucose (mg/100 mL)	350.23 ± 20	356.60 ± 55	-

1.26 in DN group versus 51.11 ± 1.92 in N group, $P = 0.05$) and moderately compared to D groups.

Discussion

The present study assessed the correlation of the serum sialic acid and NA with diabetes and nephropathy condition. In order to mimic the diabetic nephropathy condition in rats, they were injected by STZ and glycerol. NAs, also called sialidases, catalyze the removal of sialic acid residues from sialylated glycoconjugates. This enzyme is lysosomal; however, its non-lysosomal form has been found in some tissue (10). In some studies, neuraminidase activity has measured in variety of tissue including kidney and serum (11). Our results show that NA activity significantly increased in diabetic nephropathy, nephropathy, and diabetic rats compared to the control group, especially in diabetic nephropathy (Figure 2). This result indicates that diabetes and nephropathy may increase serum NA activity levels. In addition, the serum levels of NA activity were higher in diabetic nephropathy rats compared to diabetic rats with no nephropathy (Figure 2). Roozbeh et al demonstrated a significance increase in NA activity in diabetic nephropathy patients (12). In contrast, another study showed no change in the specific activity of neuraminidase in glomeruli or cortex of rats with nephrotic syndrome (13).

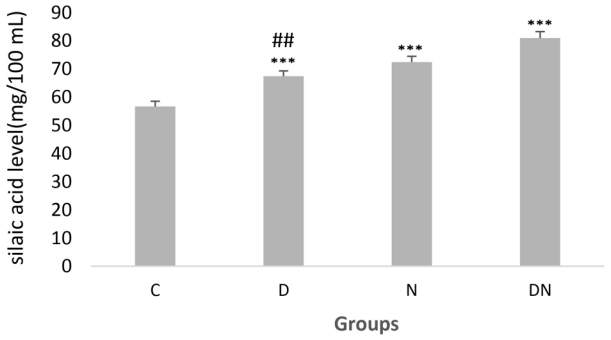

Figure 1. Sialic acid level significantly increased in D, N, DN groups compared with C. The level of sialic acid elevated remarkably in DN compared with D and N . Data are shown as mean ± S.D of at least three separate experiments. ***P<0.001 vs. C ; ## P<0.01 vs. DN. C: control; D: dibetes; N: nephropathy; DN: diabetic nephropthy.

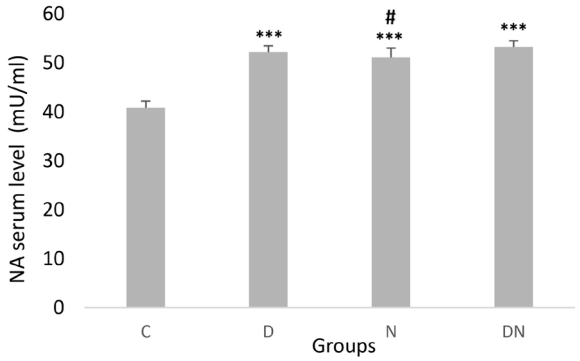

Figure 2. Neuraminidase (NA) activity level elevated significantly in D, N and DN groups compared with C. Serum neuraminidase increased considerably in the DN group compared to N. Data are shown as mean ± S.D of at least three separate experiments. ***P<0.001 vs. C; #P<0.05 vs. DN. C: control; D: dibetes; N: nephropathy; DN: diabetic nephropthy.

Another section of this study included the assessment of serum sialic acid, which has an essential role in maintaining the negative charge of glomerular basement membrane. In response to acute phase, the level of sialic acid increase in circulation due to elevated vascular permeability. Our results demonstrated that the serum sialic acid level was significantly elevated in diabetic nephropathy (Figure 1). This result is in agreement with the study by Crook et al who found serum sialic acid is significantly increased in type 2 diabetic patients (14). Izumida et al also revealed that sialic acid increased in diabetic nephropathy patients (15). Similar studies reported that serum sialic acid was significantly high in diabetic cases and remained higher when diabetes was complicated with nephropathy (15,16). The level of serum sialic acid can determine the extent of nephropathy (17), however, evidence has shown decreased cell surface sialic acid residues in hepatocytes from diabetic rats and also a reduction in sialic acid in the saliva of diabetic rats (18, 19). Various factors lead to increased sialic acid levels in the serum, including sialic acid shedding from glycoconjugates in cell membranes, increased levels of acute-phase proteins, and increased activity of NA enzyme. Serum acute-phase proteins are known to be elevated in diabetes mellitus (20). There are abnormalities in the red blood cell membrane in diabetic patients that can also lead to the release of sialic acid and increased serum sialic acid levels (15). High blood pressure, which might develop in patients with nephropathy, is a contributing factor for serum sialic acid increase because of the vascular endothelium damage and the subsequent release of sialic acid from sialyl-conjugated membrane proteins (21).

Our results show that sialic acid level is a good manifestation of diabetic complication due to the higher level in diabetic nephropathy group compared to the diabetic group. These results indicate that sialic acid is a sensitive marker for the assessment of microvascular complications in type 2 diabetes mellitus.

Conclusion

The results of the current study confirm the suggestion that serum sialic acid and possibly NA activity may be suggested as non-specific monitoring markers in the diagnosis of diabetes and its complications and nephropathy. In non-diabetic nephropathy cases, these markers may also be used as sensitive markers.

Authors' contribution

RA designed the research. FB prepared the final draft of the article. RA analyzed the data. All authors participated in conducting experiments and signed the final paper.

Conflicts of interest

The authors declare that they have no competing interest.

Ethical considerations

Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, redundancy) have been completely observed by the authors.

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