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A Distinct Profile of Serum Levels of Soluble Trail and Glycated Hemoglobin in Diabetic Nephropathy and its Relation to Different Treatment Modalities

KEYWORDS: soluble TRAIL; HbA1c; type-2 diabetes mellitus; diabetic nephropathy; biomarker discovery

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ABSTRACT *Background : TNF-related apoptosis-inducing ligand(TRAIL) was originally isolated as an inducer of apoptosis and was an important component of the immune system. Diabetic nephropathy(DN) also occurs as a late complication and chronic inflammation plays a crucial role. Although it is well acknowledged that TRAIL has an important role in Type-1(T1D) and Type-2 Diabetes(T2D) development, it is unknown whether TRAIL can inversely reflect the progression of diabetes. Our aim was to investigate the possible role of serum soluble TRAIL(sTRAIL) in DN patients. Material and Methods: We examined the association between sTRAIL and HbA1C levels, BUN, creatinin and spot urine protein were evaluated as a surrogate marker of DN in three groups of T2D patients; newly diagnosed non-treated(n=22), oral anti-diabetic drug using(n=14) and insulin-treated(n=20). Results: sTRAIL level is correlated with BUN in insulin using DN patients and HbA1C % is correlated with BUN and uric acid in oral antidiabetic drug using patients. However, there was no significant change in the sTRAIL and HbA1C levels according to the nephropathy between two patient groups that were treated differentially. Conclusion: Besides the limitation of the number of patients, our results show that sTRAIL levels showed distinctive values in DN patients depending on the treatment.*

INTRODUCTION

Diabetic nephropathy (DN) is a progressive kidney disease caused by angiopathy of capillaries in glomeruli secondary to longstanding diabetes and is the major cause of morbidity and mortality in patients with type-2 diabetes mellitus (T2DM) [1, 2]. This emphasizes the importance of interventions that help patients with type-2 diabetes to determine the risk of nephropathy.

Cell death via apoptosis is an active response of cells to altered microenvironments and is characterized by the activation of specific intracellular pathways [3]. Hyperglycemia is among the microenvironmental factors that may induce or facilitate apoptosis. A high glucose concentration, per se, promotes apoptosis in a variety of cell types, including renal tubular epithelium [4 – 6]. Lethal cytokines from the TNF superfamily activate death receptors on the cell surface with subsequent activation of caspases, central activators, and effectors of apoptosis [7 – 9]. The apoptotic process is modulated by a host of checks and balances with a multitude of positive and negative regulators [10].

The TNF-a super-family of cytokines, which comprises structurally, related proteins that play important roles in regulating cell death, immune response and inflammation. TNF-related apoptosis-inducing ligand (TRAIL), a member of the TNF super-family of cytokines, is an important component of the immune system [11]. Although it is acknowledged that it also has an important role in diabetes development, this presumed role especially on type-2 diabetes mellitus has not yet been clearly revealed.

Recent studies suggested a role for cell death in the progression of human DN. There is evidence for both destructive and especially protective roles for TRAIL in diabetes unlike other TNF-alpha family members, which are mainly known for destructive effects on pancreatic beta cells [12]. TRAIL is a type II transmembrane protein of 281 and 291 amino acids in the humans and mice, respectively, with an expected molecular mass of 33–35 kDa [13]. Membrane-bound TRAIL can be cleaved from the cell surface to form a soluble trimeric ligand that retains the proapoptotic activity [14].

Among the various molecules known to take role in the diabetes course, the recently defined Tumor-necrosis factor- (TNF-) related apoptosis-inducing ligand (TRAIL) holds a unique position. TRAIL could have an early protective role on the onset of disease in type I diabetes, whereas it could have a modulating role of the vascular complications in both type I and type II diabetes [15]. Recent studies reported that TRAIL - TRAIL receptor interaction might be an independent risk factor of progressive atherosclerosis and is a surrogate marker for peripheral artery disease [16 – 20]. Our previous study showed that the circulating soluble form of TRAIL is reduced in patients with newly diagnosed, non-drug using T2DM patients and in another study negative correlation with CRP levels in coronary artery disease was reported [21 – 22].

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There were controversial roles that attributed to TRAIL, which is an important component of the immune system. There had been many studies that the exact role of TRAIL and its receptors in the development of type-1 diabetes is yet to be identified [15, 23, 24]. However, further studies are required to clarify role of TRAIL in type-2 diabetes mellitus. In light of earlier reports, we asked whether serum soluble TNF related apoptosis inducing ligand (sTRAIL) might underlie and effect on diabetes and its' complication of diabethic nephropathy.

MATERIALS AND METHODS:

ELISA (sTRAIL)

In our study, we used the human soluble TRAIL/Apo2L ELISA kit (ab46117) for the in-vitro quantitative determination of soluble TNF-related apoptosis-inducing ligand (TRAIL) in serum samples of diabetes mellitus patients with nephropathy. The absorbance of each patient on a spectrophotometer using 450 nm and the concentration of soluble TRAIL (pg/ml) was measured.

Patients

We enrolled 22 newly diagnosed T2DM patients as control and 24 patients (mean age 48±8.2 years, %46 male and %54 female) with DN according to presence of microalbuminuria (30 to 300 mg albumin/24 hours or albumin to creatinine ratio [ACR] of 3.4 to 34.0 mg/mmol [30 to 300 mg/g] or macroalbuminuria (>300 mg albumin/24 hours or ACR >34 mg/mmol [300 mg/g]. And none of the patients had any infectious disease, other autoimmune disease or cancer.

All patients gave their informed, written consent. The study was approved by the local independent ethics committee and was performed in accordance with the ethical principles of the Declaration of Helsinki.

Statistical Analysis

The statistical package for the Social Sciences 13.0 software for Windows (SPSS Inc., Chicago, III) and GraphPad Prism version 5 (La Jolla, CA, USA) were used to plot the data and perform statistical analyses. A non-parametric unpaired student's T test was used to evaluate sTRAIL levels in patient groups versus control. All correlation analyses used Spearman's Rho tests.

RESULTS

The demographics of the analyzed patients were summarized in Table 1.

The mean serum sTRAIL level in the newly diagnosed T2DM patients was 989.6 pg/ml; in oral anti-diabetic drug using T2DM patients was 895.09 pg/ml and 944.81 pg/ml in the insulin treated patients. According to the serum sTRAIL level, we observed that the treatment modality has no effect on the apoptotic marker sTRAIL. (Figure IA). The HbA1C levels were also evaluated in T2DM patients; 6.40 ± 0.28 % in newly diagnosed T2DM patients, 7.40 ± 0.42 % in oral antidiabetic treated patients and 8.59 ± 0.39 % in insulin using patients. However, the HbA1C % in both groups was not statistically different as shown in Figure IB.

Also we observed that the levels of sTRAIL and HbA1c were in independence of clinical presentations of type-2 diabetes mellitus late complication of DN. We investigated evidence for a correlation between serum sTRAIL levels, HbA1c levels and the severity of nephropathy by evaluating spot urine protein, BUN, creatinin and uric acid. The sTRAIL levels did not correlate with any of these markers in oral antidiabetic drug using DN patients (Supp. table IA). However, there is a correlation between sTRAIL and BUN levels of DN patients (p=0,0161) (Supp. table IB). Moreover, we compare the HbA1C levels and other clinical markers in both treatment modalities. While there is a correlation between HbA1C % and BUN (p=0,0044) and uric acid (p=0,0242) levels in oral antidiabetic drug treated patients with DN (Supp. table IIA), there is no any correlation in insulin treated DN patients (Supp. table IIB).

DISCUSSION

TRAIL is normally expressed in many human tissues including kidney, suggesting that TRAIL must not be cytotoxic to most tissues in vivo under normal physiological conditions. However, when normal cells are immersed in an inflammatory environment, data from knockout mice suggest that TRAIL may induce parenchymal cell apoptosis.

Most TRAIL literature is referred to its potent tumor cell-killing activity. Different combinations of TRAIL and chemotherapeutic drugs or the use of agonistic anti-TRAILR1 or R2 antibodies shows promising results in the treatment of renal carcinoma. However, TRAIL also has non-apoptotic functions, such as pro-survival and proliferative effects. In normal kidney, TRAIL is expressed only in tubules and absent from glomeruli. TRAIL-R1 has a similar pattern of expression to TRAIL, while TRAILR2 is additionally expressed in Henle's loop. TRAILR3 expression was not detected in the normal kidney, and there are no reports regarding renal tissue expression of TRAIL-R4. No kidney pathology has been reported in TRAIL knockout mice, suggesting that TRAIL is not required for normal kidney development and physiology.

Previous studies demonstrated that circulating soluble TRAIL levels were significantly lower in newly diagnosed, non-drug using type-2 diabetes mellitus patients than the healthy individuals. However, there was no correlation between sTRAIL levels and other biochemical biomarkers like HbA1C, blood glucose level and BMI; therefore, its prognostic value is limited.

Also the most recent studies reported that variable serum sTRAIL levels have been observed in many disorders like cancer, cardiac, renal and even in allergic diseases [25 – 28]. Furthermore, since the biological effects of TRAIL are known, to be largely receptor and cell type-specific in autoimmune diseases like diabetes and rheumatoid arthritis, and also serum osteoprotegerin indicated as a marker for the severity of DN [28 – 30]. In another recent study, the association of sTRAIL levels with atherosclerosis in patients with type 2 diabetes mellitus was examined. However, sTRAIL was not useful to evaluate atherosclerotic lesions [17].

In conclusion, to the best of our knowledge, this is the first study to assess serum sTRAIL and HbA1c levels in T2DM patients with late complication of DN and to evaluate the proteins' relationship with clinical status. Our study demonstrates that sTRAIL levels are not changing between different treatment modalities as oral anti-diabetic drugs or insulin in DN patients. However, it is correlated with BUN levels of insulin using DN patients. And HbA1C % is correlated with BUN and uric acid levels in oral anti-diabetic drug using patients.

Further studies are necessary to investigate whether the TRAIL system has a role or the role of sTRAIL as a marker in type-2 diabetes mellitus and its complications.

FIGURES AND LEGENDS

Table I: Demographics and laboratory findings of the newly diagnosed (Group I) and diabetic nephropathy patients divided in two groups according to their treatment modalities; oral antidiabetic drug using as group II and insulin treated patients as group III. n: number of patients enrolled.

Group	Gender (male:female)	Age	Spot Urine Protein	BUN	Creatinin	Uric Acid
I (n=22)	5 : 17	54.2	-	-	-	-
II (n=14)	7 : 7	57.6	1054	25.6	1.1	5.8
III (n=20)	13 : 7	59.4	2026	27.9	1.4	6.1

Supplementary Table II: The correlation analysis between HbA1C level and spot urine protein, BUN, creatinin and uric acid levels of oral antidiabetic drug (IIA) and insulin (IB) using diabetic nephropathy patients - Spearman Rho correlation analysis.

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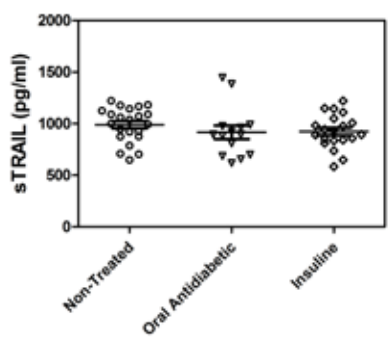
IIA

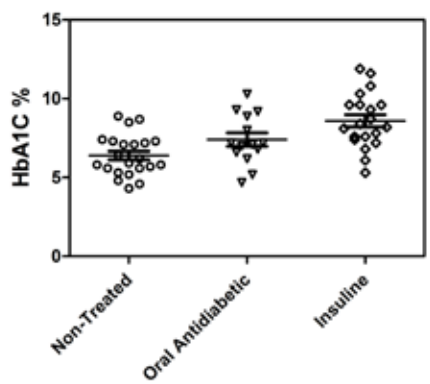
Oral Antidiabetic (n=14)	HbA1C Level & Spot Urine Protein	HbA1C Level & BUN	HbA1C Level & Creatinin	HbA1C Level & Uric Acid
Spearman r	-0,06601	-0,7107	-0,5222	-0,5969
95% confidence interval	-0.5880 to 0.4949	-0.9046 to -0.2729	-0.8299 to 0.02923	-0.8609 to -0.07960
P value	0,8226	0,0044	0,0554	0,0242
Is the correlation significant? (alpha=0.05)	No	Yes	No	Yes

IIB

Insulin Treatment (n=20)	HbA1C Level & Spot Urine Protein	HbA1C Level & BUN	HbA1C Level & Creatinin	HbA1C Level & Uric Acid
Spearman r	-0,05275	-0,08875	-0,05520	-0,3023
95% confidence interval	-0.4948 to 0.4109	-0.5216 to -0.3804	-0.4966 to 0.4089	-0.6649 to -0.1756
P value	0,8252	0,7098	0,8172	0,1952
Is the correlation significant? (alpha=0.05)	No	No	No	No

Figure I: Scatter dot plots of peripheral blood samples from 14 oral anti-diabetic drug treated T2DM patients and 20 insulin using T2DM patients, showing the sTRAIL levels (pg/ml) (IA) and HbA1C (%) (IB).





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