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Original Article

Relationship between plasma levels of soluble receptor for advanced glycation end-products and tumor necrosis factor-alpha in diabetic nephropathy

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Abstract

Diabetic nephropathy (DN) is a common microvascular complication of type II diabetes mellitus (T2DM) involving inflammatory kidney damage. Excessive signaling via the receptor for advanced glycation end-products (RAGE) plays an important role in mediating renal inflammation. A soluble form of RAGE (sRAGE) is an important regulator of the RAGE signaling pathway. This study aimed to evaluate the relationship between sRAGE and TNF-alpha in DN. A comparative study was carried out comprising three groups (Control group, T2DM group, and DN group). There were 35 participants in each group. The study participants' plasma levels of sRAGE and TNF-alpha were quantified using enzyme-linked immunosorbent assays. Plasma levels of TNF-alpha were significantly higher in T2DM and DN compared to control ($P<0.0001$). Also, plasma levels of sRAGE were significantly lower in T2DM and DN compared to control ($P<0.0001$). Furthermore, there was a significant correlation between sRAGE and TNF-alpha levels in T2DM ($r=-0.3$; $P<0.05$) and also DN ($r=-0.37$; $P<0.001$). These results indicate that sRAGE impairment may be responsible for TNF-alpha elevation in T2DM and DN.

Keywords: type II diabetes, nephropathy, soluble receptor for advanced glycation end-products, tumor necrosis factor.

Introduction

Type II diabetes mellitus (T2DM) is a major public health concern that affects nearly 6.28% of the world's population [1]. The current efforts to reduce the progression from metabolic syndrome and prediabetes to diabetes have failed to produce any impact. Nearly 40% of T2DM patients eventually develop diabetic nephropathy (DN), which involves microvascular complications of the kidneys [2]. The majority of DN patients succumb to death due to end-stage renal disease, cardiovascular disease, or infection [2].

Given the public health magnitude, there is an urgent need to understand the pathophysiological mechanism that drives the progression of T2DM into DN. This information may unlock novel drug targets for ameliorating the progression. The major pathological changes in the DN kidneys are glomerular and tubular membrane thickening [3]. Hemodynamics, metabolic pathways, and inflammatory pathways are involved in the pathophysiology of DN [4]. The advanced glycation end product (AGE) pathway is one of the metabolic pathways that proved to play an important role in DN pathogenesis. Advanced glycation end products are conjugates of sugars with proteins and lipids. AGE formation is substantially higher in T2DM due to hyperglycemia [4].

AGE is a potent activator of inflammatory cell signaling. AGE binds to its cognate receptor called receptor

for the advanced glycation end product (RAGE). The RAGE receptor is expressed in various types of cells like endothelial cells, neurons, mononuclear phagocytes, and cardiac myocytes, smooth muscle cells [5]. AGE-RAGE interaction activates RAGE signaling via the NF-kB pathway resulting in the secretion of inflammatory cytokines [5]. Over-activation of the RAGE signaling pathway is an important reason for the increased levels of inflammatory cytokines in DN [6]. Recent studies have shown that the regulators of the RAGE signaling pathway are altered in DN [7]. This indicates that abnormal changes in the regulation may also be responsible for excessive RAGE signaling in DN.

The soluble form of the RAGE receptor (sRAGE) is an important regulator of RAGE signaling. sRAGE is produced from the cell surface RAGE due to the proteolytic cleavage of the extracellular domain (Figure 1). sRAGE retains the capacity for AGE binding but cannot induce intracellular cell signaling (Figure 1).

sRAGE competes with cell surface RAGE for AGE ligands. This limits the amount of AGE ligand reaching the cell surface RAGE [5]. Therefore, diminished levels of sRAGE may contribute to excessive RAGE signaling. This study aimed to evaluate the relationship between sRAGE and TNF alpha in DN.

Material and methods

Study design

This was an observational study involving a comparison of three groups. Group 1 comprised healthy volunteers (Control); Group 2 comprised patients with type II diabetes mellitus (T2DM); Group 3 comprised patients with type II diabetes mellitus with renal complication (DN). Each group consisted of 35 participants. All the study participants were recruited from R. L. Jalappa Hospital and Research Centre, Kolar, Karnataka, India. The study period was from April 2019 to April 2020. Ethical clearance was obtained and approved by the Institutional Ethics Committee of Sri Devaraj Urs Medical College, Kolar, Karnataka, India (SDUMC/KLR/IEC/218/2018-19). Study participants were enrolled after obtaining written informed consent.

Inclusion and exclusion criteria

Participants were diagnosed with T2DM based on the criteria of the Indian Council of Medical Research [8]. Diabetic nephropathy was staged based on the guidelines of the Joint Committee on Diabetic Nephropathy [9]. The inclusion and exclusion criteria for each study group are summarised in Table 1.

Estimation of sRAGE and TNF-alpha levels

Blood samples (3 ml) were collected from each study participant in a sterile EDTA vacutainer. The plasma was separated by centrifuging the samples at 3000 rpm for 5 min. The plasma was then transferred into a sterile 1.5 ml microcentrifuge tube and stored at -80°C until further use. The stored serum samples were then used to assess the levels of TNF alpha and sRAGE. Commercially available enzyme-linked immunosorbent assay kits were used to estimate sRAGE levels (Cat # SEA645Hu, Cloud-Clone Corp., USA) and TNF-alpha (Cat # SEA133Hu, Cloud-Clone Corp., USA).

Statistical analysis

Statistical analysis was conducted using GraphPad Prism 5 (GraphPad Software Inc, San Diego, California). The data were checked for normal distribution using the Shapiro-Wilk test. If the data followed a normal distribution, then parametric tests were used; otherwise, non-parametric tests were used. The P-value was considered significant if it was less than 0.05.

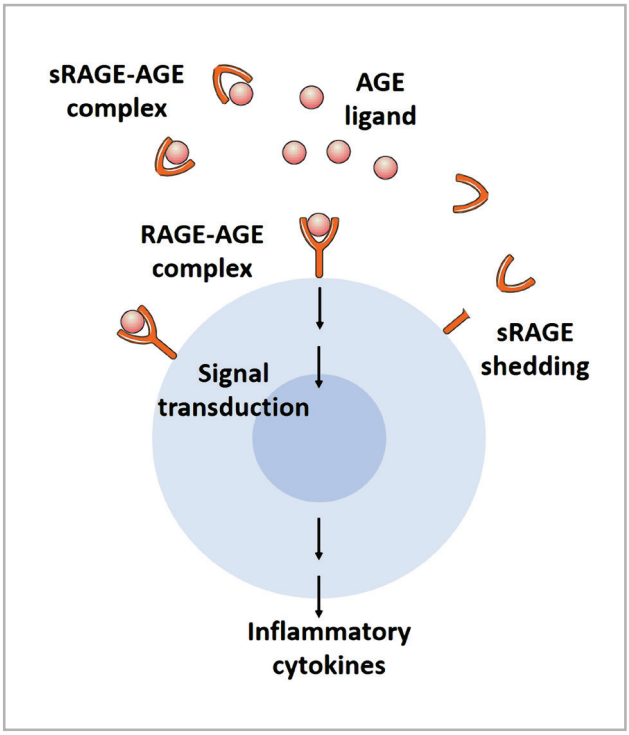

Figure 1: Generalised representation of the RAGE pathway activation, regulation and response in the human cell.

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Table 1: Inclusion and exclusion criteria for selection of study participants.

Parameter	Control	T2DM	DN
Inclusion criteria			
Age (Years)	>18	>18	>18
Gender (Male/Female)	Both	Both	Both
HbA1c (%)	<5.7	>6.5	>6.5
Serum creatinine (mg/dL)	0.6–1.3	>1.3	>1.3
eGFR (ml/min/1.73 m^3)	>90	>90	<15
Blood urea nitrogen (mg/dL)	6–24	6–24	>24
Fundus	Normal	Normal	Normal
Exclusion criteria			
Pregnant and lactating women	Excluded	Excluded	Excluded
History of any chronic illness	Excluded	Excluded	Excluded

Results

Clinical profile of the study participants: A total of 105 participants were included in this study. The measures of glycaemic control and renal function of the study participants are summarised in Table 2.

Plasma levels of sRAGE in the study groups: The plasma sRAGE levels in the study groups showed normal distribution. Therefore, mean and standard deviation were calculated, and parametric tests were used for the comparison.

The average plasma sRAGE levels in the control, T2DM, and DN groups were 336.9 pg/mL, 266.1 pg/mL, and 254.4 pg/mL, respectively (Figure 2). The sRAGE levels in the three groups were significantly different (P<0.0001; ANOVA). Next, the difference between the two groups was compared. The sRAGE levels were significantly lower in T2DM compared to control (P<0.0001; Unpaired t-test). Also, the sRAGE levels were significantly lower in DN compared to the control (P<0.0001; Unpaired t-test). Furthermore, there was no significant difference in the sRAGE levels of T2DM and DN groups (P=0.12; Unpaired t-test).

Plasma levels of TNF-alpha in the study groups: The plasma TNF-alpha levels in the study groups did not show normal distribution. Therefore, median and interquartile ranges were calculated, and non-parametric tests were used for the comparison.

The average plasma TNF-alpha in the control, T2DM, and DN groups were 104.4 pg/mL, 189.1 pg/mL, and 193.5 pg/mL, respectively (Figure 3). The TNF-alpha levels in the three groups were significantly different (P<0.0001; Kruskal Wallis test). Next, the difference between the two groups was compared (Figure 3). The TNF-alpha levels were significantly higher in T2DM compared to control (P<0.0001; Mann-Whitney U test).

Table 2: Clinical and demographic profile of the study participants.

Parameters	Control	T2DM	DN
Age (years)	55.89±7.39	54.69±7.42	55.06±7.8
Gender (Male/Female %)	42.86/57.14	42.86/57.14	48.57/51.43
FPG (mg/dL)	87.15±8.08	153.33±19.32	149.69±22.43
HbA1c (%)	4.65±0.29	8.84±0.52	7.61±0.60
Serum creatinine (mg/dL)	0.74±0.10	0.74±0.10	3.30±0.65
eGFR (ml/min/1.73 m^3)	98.83±11.95	101.06±9.14	18.66±4.99
Blood urea nitrogen (mg/dL)	15.04±3.24	14.89±3.40	49.34±16.26

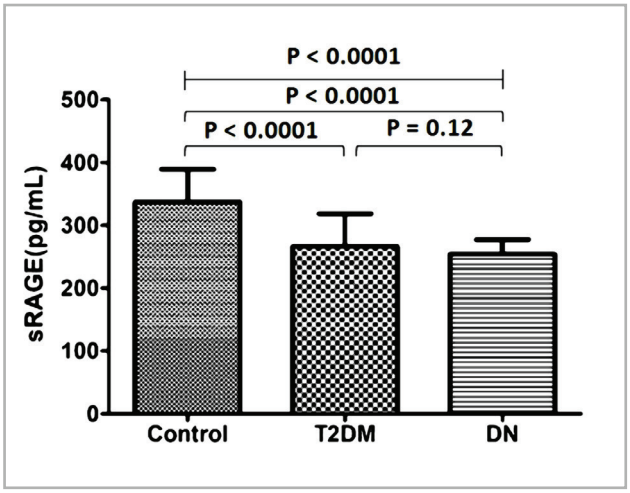

Figure 2: Plasma levels of sRAGE in the study groups.

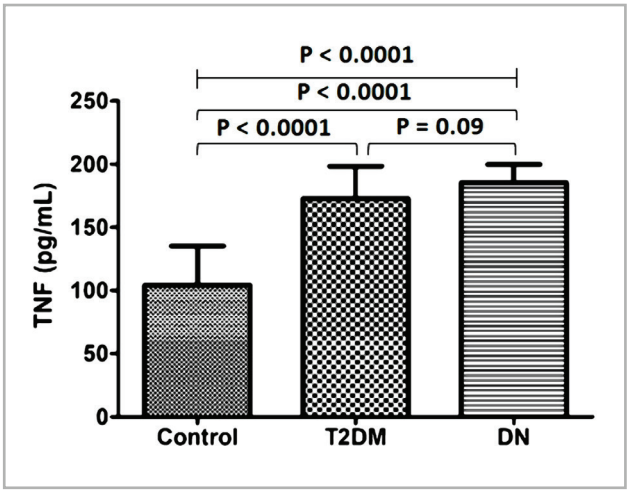

Figure 3: Plasma levels of TNF-alpha in the study groups.

Also, the TNF-alpha levels were significantly higher in DN compared to control (P<0.0001; Mann-Whitney U test). However, there was no significant difference in the TNF-alpha levels of the T2DM and DN groups (P=0.09; Mann-Whitney U test).

Relationship between serum levels of sRAGE and TNF-alpha: The results are shown in Figure 4 A–C. There was a significant inverse correlation between sRAGE and TNF-alpha levels in the T2DM group (r=-0.3; P<0.05; Spearman's correlation test) and DN group (r=-0.37; P<0.001; Spearman's correlation test). However, no significant correlations were observed in the control group (r=-0.018; P=0.9; Spearman's correlation test).

Discussion

This study aimed to evaluate the relationship between TNF alpha and sRAGE in diabetic nephropathy. The major findings of this study are that (i) plasma TNF-alpha is elevated in both T2DM and DN, (ii) sRAGE is reduced in both T2DM and DN, and (iii) TNF-alpha showed reciprocal correlation with sRAGE. These results indicate that sRAGE impairment may be responsible for TNF-alpha elevation in T2DM and DN. To the best of our knowledge, this is the first study from India to show that sRAGE may be involved in regulating TNF-alpha levels in T2DM and DN.

Nakamura *et al.* first demonstrated the reduction of sRAGE in the serum of T2DM patients [10]. Several studies carried out since then have reported inconsistency in the relationship. The study carried out by Grossi *et al.* failed to see any difference in the blood sRAGE levels between controls and T2DM patients [11]. However, a significant difference was seen when T2DM patients also had microvascular complications [11]. These observations indicated that sRAGE might have a protective role against the development of microvascular complications in T2DM. The studies of Farhan *et al.* showed that sRAGE was substantially lower in both T2DM and DN groups compared to the control [12]. Furthermore, they also observed a significant difference between T2DM and DN. In this study, plasma sRAGE was observed to be reduced in both T2DM and DN; however, there was no significant difference between the two groups. The results of this study indicate sRAGE is reduced in T2DM but does not indicate any protective role against the development of DN.

The involvement of TNF-alpha in DN pathogenesis was demonstrated nearly three decades ago in an animal model study [13]. Since then, elevated TNF-alpha levels have been shown in the serum and urine of DN patients. A meta-analysis of nine case-control studies showed that elevation of TNF-alpha is higher in DN compared to T2DM [14]. However, the results of this study are contrary to the findings of the meta-analysis. In this study, TNF-alpha levels were more or less similar in both DN and T2DM groups. A similar trend was observed in a recent study [15]. In addition, a study comparing TNF-alpha levels in the three stages of DN found no significant difference between the groups [16]. Another study found a positive correlation between DN and TNF-alpha in the urine samples but not with serum samples [17]. The blood levels of TNF-alpha appear to be elevated T2DM but do not appear to be augmented by progression to DN.

In this study, the reduction of plasma sRAGE and elevation of plasma TNF-alpha showed reciprocal correlation. This indicates that reducing sRAGE may be

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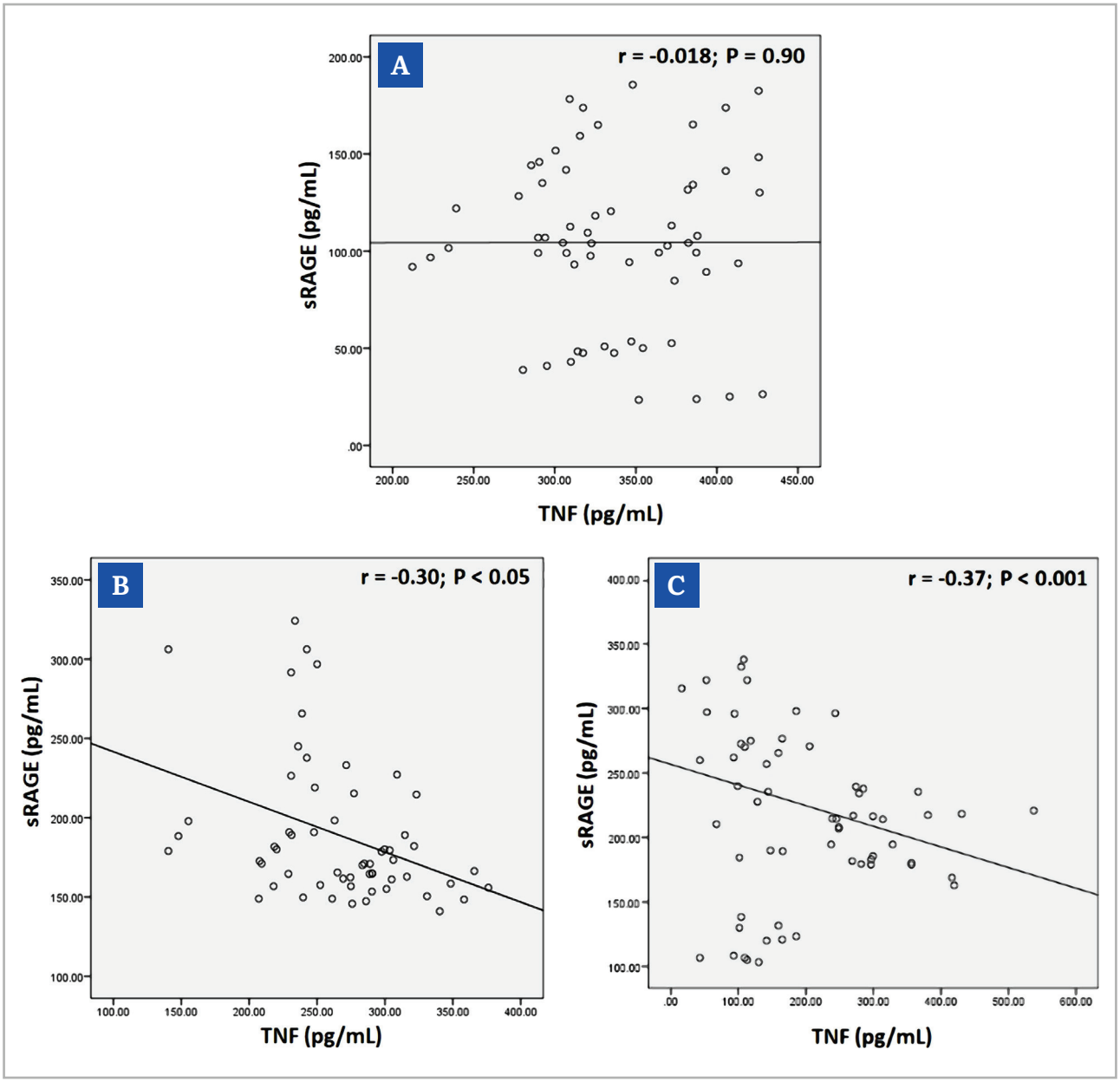

Figure 4: Correlation between the study groups' plasma levels of sRAGE and TNF-alpha. A – Control; B – T2DM; C – DN.

involved in regulating plasma TNF-alpha levels. A similar relationship was also reported by Nakamura *et al.* [10.] However, the strength of correlation observed in this study was weak, indicating that factors other than sRAGE may also be involved in regulating plasma TNF-alpha in T2DM and DN.

Conclusion

Overall, the results of this study support the conclusion that sRAGE may be one of the factors responsible for regulating plasma TNF-alpha in T2DM. However, it is unlikely to be involved in the progression to DN.

Conflict of interest

The authors declare no conflict of interest.

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