Fractalkine in type 2 Egyptian diabetics with and without nephropathy

Ebtissam Zakaria[a], Hoda Al-Rawi[a], Nashwa S. Ghanem[a], Naglaa M. Elsayed[a] and Laila A. Rashed[b]

Departments of [a]Internal Medicine, Diabetes & Endocrinology and [b]Medical Biochemistry, Faculty of Medicine, Cairo University, Cairo, Egypt

Correspondence to Naglaa M. Elsayed, 3 Abou-elnaga St., Madkour, Faisal, Giza, Egypt
Tel: +01148948023;
e-mail: Naglaamhd1@yahoo.com

Received 11 April 2013
Accepted 5 May 2013

The Egyptian Society of Internal Medicine
2013, 25:133–136

Background
Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Diabetic nephropathy is the major microvascular complication of diabetes mellitus; one of the earliest clinical signs of diabetic nephropathy is an elevated urinary albumin excretion (UAE), referred to as microalbuminuria. Fractalkine is a large cytokine protein of 373 amino acids; it contains multiple domains. Fractalkine (CX3CL1) is a unique chemokine and the only representative of the CX3C group. It exists as a membrane-bound and soluble form. It interacts with cells expressing CX3CR1, a G-coupled protein receptor. It is also commonly known by the names fractalkine (in humans) and neurotactin (in mice).

Aim
Our study aimed to assess fractalkine levels in type 2 Egyptian diabetic patients with and without diabetic nephropathy and its role as a marker in the development of diabetic nephropathy.

Patients and methods
Our study was carried out on 75 individuals: 25 controls, 25 type 2 diabetic patients without diabetic nephropathy, and 25 type 2 diabetic patients with diabetic nephropathy. These patients were subjected to a full laboratory workup including fasting and postprandial blood glucose, glycated hemoglobin A1C, serum urea and creatinine, 24-h UAE, and fractalkine level.

Results and conclusion
Our study showed that the serum fractalkine concentration was significantly elevated in type 2 diabetic patients with nephropathy (1153.14 ± 261.1) compared with type 2 diabetic patients without nephropathy (705.78 ± 150.59) and the control group (251.5 ± 64) (both $P=0.000$). There was a significant correlation between serum fractalkine level and 24-h UAE, HBA1C, and serum creatinine. Thus, this positive correlation between serum fractalkine level and UAE could be an early predictor of microvascular complications in diabetic patients. We can conclude that serum fractalkine plays a pathogenic role in the development of diabetic nephropathy.

Keywords:
diabetes, diabetic nephropathy, fractalkine, urinary albumin excretion

Egypt J Intern Med 25:133–136

1110-7782

Introduction

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both [1]. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels [2]. Diabetic nephropathy is a major microvascular complication of diabetes mellitus [3]. One of the earliest clinical signs of diabetic nephropathy is elevated urinary albumin excretion (UAE), referred to as microalbuminuria [4].

Fractalkine, also known as CX3CL1 or neurotactin, is the only known member of the CX3C or the delta chemokine subfamily [5]. It exists as a membrane-bound and soluble form. It interacts with cells expressing CX3CR1, a G-coupled protein receptor. Fractalkine is not only a chemotactic factor but also participates in leukocyte trafficking, adhesion and cytotoxic activities, modulates the expression of cytokines, adhesion molecules, and free oxygen radicals, iNOS, and influences apoptosis [5].

Fractalkine is induced on activated endothelial cells and promotes strong adhesion of T cells and monocytes through its receptor CX3CR1. In the kidney, fractalkine expression might be induced by high shear stress and plays an important role in prolonged glomerular diseases. Fractalkine in diabetic kidneys was detected on glomerular capillaries and the mesangium. This upregulation was suppressed by treatment with angiotensin-converting enzyme inhibitor (ACEI) or aminoguanidine. High glucose levels, AGE formation, and cytokine activation in diabetes may induce fractalkine upregulation

 DOI: 10.7123/01.EJIM.0000432173.94048.48

in the kidneys and lead to progression of diabetic nephropathy [6]. The fractalkine system may promote renal inflammation and renal fibrogenesis [7].

Aim

This study aimed to assess fractalkine levels in type 2 Egyptian diabetic patients with and without diabetic nephropathy and the role of fractalkine as a marker in the development of diabetic nephropathy.

Patients and methods

This study was carried out at Cairo University, Kasr Al Aini Outpatients Diabetic Clinic, in the period from (December 2011 to December 2012). The study was carried out on 75 individuals ranging in age from 42–73 years; 32 patients were men (42.7%) and 43 were women (57.3%). They were classified into three groups: group A included 25 healthy individuals as controls; group B included 25 type 2 diabetic patients without diabetic nephropathy; and group C included 25 type 2 diabetic patients with nephropathy (confirmed by full medical history and laboratory studies).

Exclusion criteria

(a) Type 1 diabetes mellitus and (b) history of treatment with ACEIs.

All participants were subjected to the following: assessment of detailed medical history, full physical examination, and determination of fasting and postprandial blood glucose, serum urea and creatinine level, and glycated hemoglobin (HBA1c). Twenty-four hour UAE and serum fractalkine level were determined using the quantitative sandwich enzyme immunoassay technique.

The protocol of this study was first approved by the scientific board of Internal Medicine department; verbal consent was taken from all studied groups.

Data were coded and patient names or identity was concealed in data collection forms or during statistical analysis.

Statistical analysis

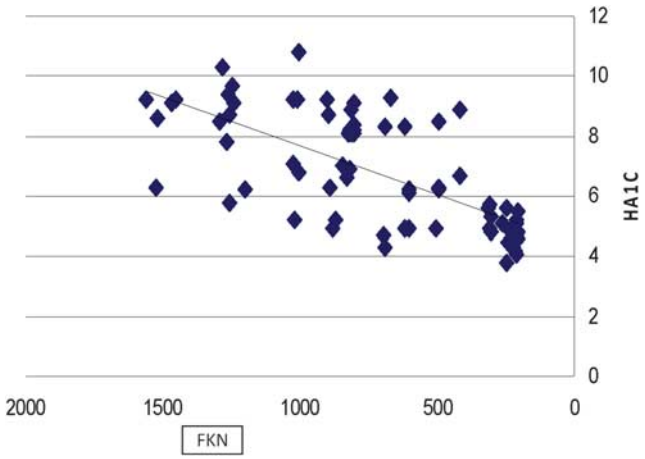
The data were coded and entered using the statistical package SPSS (SPSS Inc., Chicago, Illinois, USA), version 15. The data were summarized using descriptive statistics: mean, SD, minimal and maximum values for quantitative variables, and number and percentage for qualitative values. The relationship between fractalkine and the different measured parameters was determined using the bivariate correlation with the Pearson correlation coefficient.

Results

Figure 1 shows a strong positive correlation between serum fractalkine level and HA1c in the groups studied ($P = 0.000$).

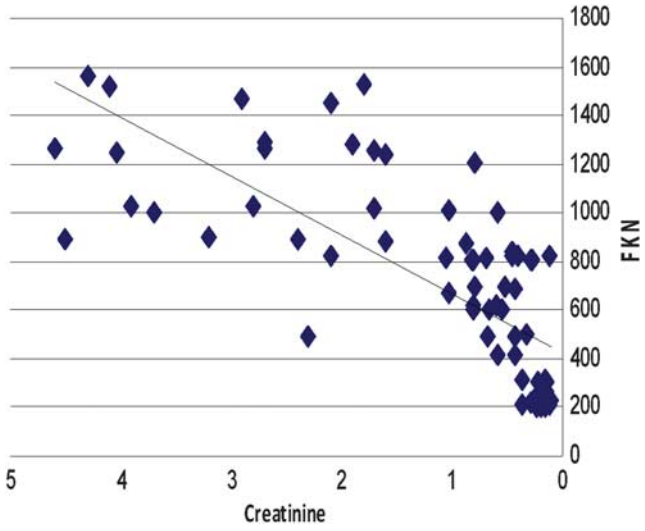
Figure 2 shows a strong positive correlation between serum fractalkine level and serum creatinine level in the groups studied ($P = 0.000$).

Figure 1



Correlation between serum fractalkine (FKN) level and HA1c among the groups studied.

Figure 2



Correlation between serum fractalkine (FKN) level and creatinine among the groups studied.

Figure 3 shows a strong positive correlation between serum fractalkine level and 24-h UAE in the groups studied ($P = 0.000$) (Tables 1 and 2).

Discussion

Diabetic nephropathy is a major microvascular complication of diabetes mellitus. Fractalkine, also known as CX3CL1 or neurotactin, is the only member of the CX3C or the delta chemokine subfamily [5]. One of the earliest clinical signs of diabetic nephropathy is an elevated UAE, referred to as microalbuminuria [4].

Studies have been carried out to investigate the possibility of using fractalkine as a predictor of microvascular complications in type 2 diabetic patients.

This study showed that serum fractalkine concentration was significantly elevated in group C (type 2 diabetic patients with diabetic nephropathy) compared with type 2 diabetic patients without nephropathy and the control group.

In this study, a correlation was found between serum fractalkine level and the other parameters including microalbuminuria, HA1c, and serum creatinine in the groups studied.

This observation may be supported by the finding of Kikuchi *et al.* [8], who reported fractalkine and CX3CR1 upregulation in an early stage of diabetic kidney, which suggested that fractalkine expression and CX3CR1-positive cell infiltration in diabetic kidneys might play an important role in the progression of diabetic nephropathy.

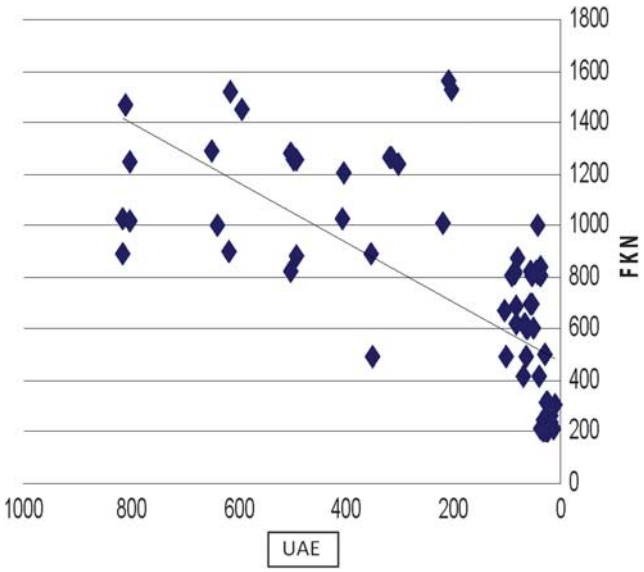
In the same study carried out by Kikuchi [8], it was found that high glucose levels, AGE formation, and cytokine activation in diabetes may induce fractalkine upregulation in the kidneys and lead to progression of diabetic nephropathy, which supports the results of our study.

In 2004, Kikuchi *et al.* [9] reported Fractalkine and CX3CR1 upregulation in an early stage of diabetic kidney. This upregulation, as well as UAE, was suppressed by treatments with temocapril and aminoguanidine for 8 weeks. These findings suggest that fractalkine expression and CX3CR1-positive cell infiltration in diabetic kidneys might play an important role in the progression of diabetic nephropathy. Some CX3CR1-positive cells were ED3 positive. Thus, these findings suggest that fractalkine expression and CX3CR1-positive cell infiltration in diabetic kidneys might play an important role in the progression of diabetic nephropathy.

Another study carried out by Haskell *et al.* [7] found that, in folic acid nephropathy, there is a good correlation between the expression of CX_3C-L with markers of interstitial inflammation and fibrosis that may result from upregulation by proinflammatory and profibrotic cytokines as well as by reactive oxygen species in tubular epithelial cells. The fractalkine system may promote renal inflammation and renal fibrogenesis.

Another study carried out by Jerath *et al.* [10] concluded that atherosclerosis is an inflammatory process that is strongly affected by chemokines that regulate the trafficking of inflammatory cells. In humans, genetic studies have shown that specific polymorphisms in chemokine and chemokine receptor genes are associated with atherosclerotic diseases including coronary artery disease and carotid artery occlusive disease. Specifically, there are sound data supporting roles for the following receptors and their ligands: CCR2 and MCP-1 (CCL2);

Figure 3



Correlation between serum fractalkine (FKN) level and urinary albumin excretion (UAE) among the groups studied.

Table 2 Comparison of fractalkine in group A (control), group B (no nephropathy), and group C (nephropathy)

	Group A	Group B	Group C
Fractalkine	251.5 ± 64	705.78 ± 150.59	1153.14 ± 261.1
P value	–	0.000	0.000

Table 1 Laboratory workup performed in the three groups included in the study

	FBS	2HPP	Creatinine (mg/dl)	HA1c (%)	UAE (mg/24 h)	FKN (pg/ml)
Control						
N	25	25	25	25	25	25
Mean	87.9	120.4	0.6032	4.8320	25.9520	251.4960
SD	9.44	11.4	0.2920	0.48795	6.25041	64.00489
No nephropathy						
Mean	139.6	320.4	0.6064	7.2136	63.9040	705.78
SD	34	55.9	0.23512	1.53773	19.97871	150.58
Nephropathy						
Mean	170	255.6	2.6468	8.0560	508.8840	1153.14
SD	36.1	58.6	1.11941	1.65984	201.38792	261.104
Total						
N	75	75	75	75	75	75
Minimum	69	100	0.6	3.8	10.8	206.4
Maximum	251	390	4.6	10.8	816.2	1562
Mean	132.54	202	1.1531	6.7005	199.5800	703.4733
SD	44.6	75.2	1.25850	1.90442	249.03810	410.0275

2HPP, 2 hour post prandial; FBS, fasting blood sugar; FKN, fractalkine; UAE, urinary albumin excretion.

CX3CR1 and fractalkine (CX3CL1); CCR1, CCR5, and RANTES (CCL5); CXCR2 and IL-8 (CXCL8); CXCR6 and CXCL16; and CXCR3 and its ligands MIG (CXCL9), IP-10 (CXCL10), and I-TAC (CXCL11). These chemokines and their receptors participate in vascular inflammation through T-cell and monocyte chemoattraction, adhesion of monocytes to the vessel wall, and vascular smooth muscle cell migration and proliferation. Chemokines and chemokine receptors are being studied as potential therapeutic targets for the prevention or retardation of atherosclerotic disease. Although many of these therapies are promising, there are also limitations to specific chemokine-targeted therapy.

A study carried out by Umehara *et al.* [11] found that accumulating evidence that fractalkine is expressed on endothelial cells during glomerulonephritis and cardiac allograft rejection, as well as on cardiac endothelial cells activated by proinflammatory cytokines, might provide an insight into the pathogenesis of vascular injury. Here, he propose a model in which fractalkine mediates vascular injury through the accumulation and activation of killer cells [11].

Some researchers have reported that the majority of leukocytes infiltrating the kidney in human renal diseases express CX3CR1, supporting the importance of this molecule in the clinical setting. The reduction of interstitial accumulation of mononuclear cells in albumin-loaded mice treated with anti-CX3CR1 antibodies was associated with amelioration of renal function. Thus, CX3CR1 might help to direct mononuclear cells into the peritubular interstitium and increase their adhesion, which in turn favors interstitial inflammation and disease progression [12].

As fractalkine is expressed on activated endothelial cells and its receptor, CX3CR1, is expressed on NK cells, monocytes, and some $CD8^+$ T cells, all of which possess cytolytic function, regulatory dysfunction of fractalkine expression may well be involved in inflammatory conditions leading to vascular injury. Fractalkine activates NK cells, resulting in enhanced cytolysis of fractalkine-expressing endothelial cells. As endothelial cells are primary targets of immunologic attack, fractalkine seems to be involved in the pathogenesis of vascular injury [13].

It has also been reported that fractalkine (CX3CL1) induces arrest of CD16– monocytes under flow conditions; therefore, it might be possible that within renal tissues, fractalkine functions as an arrest chemokine and serves as one of the factors that induce Monocyte adhesion preceding migration into diabetic kidney. During the inflammatory process in the diabetic kidney [14], increased CX3CR1 mRNA expression was detected in an early stage of diabetic kidney, and some CX3CR1-positive cells seem to be activated macrophages [8]. The expression of CX3CR1 by T lymphocytes under different inflammatory conditions was reported [15].

Conclusion

In this study, we found that the serum fractalkine concentration was significantly elevated in type 2 diabetic patients with diabetic nephropathy compared with type 2 diabetic patients without nephropathy and the control group. There was a significant correlation between serum fractalkine level and UAE, HBA1C, and serum creatinine. We reported that serum fractalkine level may be used as a good predictor for renal insufficiency and could be a predictor of microvascular complications in type 2 diabetic patients.

Recommendation

We recommend further studies to confirm our results and to measure the levels of the soluble and membrane-bound forms of fractalkine and their significance, to detect further early predictors of diabetic nephropathy in type 2 diabetic patients as well as the role of other risk factors of the metabolic syndrome and their synergistic role in diabetes mellitus in early kidney damage, to assess the possibility of using fractalkine as a novel future biomarker in patients with diabetic nephropathy.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

References

1 Wold LE, Ceylan Isik AF, Ren J. Oxidative stress and stress signaling: menace of diabetic cardiomyopathy. Acta Pharmacol Sin 2005; 26:908–917.

2 American Diabetes Association. Standards of medical care in diabetes–2008. Diabetes Care 2008; 31 (Suppl 1): S12–S54.

3 Buse PE. Type 1 diabetes mellitus. In: Kronenberg HM, Melmed S, Polonsky KS, Larsen PR, editors. *Kronenberg: Williams textbook of endocrinology*. 11th ed. Philadelphia, Pa: Saunders, Elsevier; 2008.

4 Gross JL, De Azevedo MJ, Silveiro SP, Canani LH, Caramori ML, Zelmanovitz T. Diabetic nephropathy: diagnosis, prevention and treatment. Diabetes Care 2005; 28:164–176.

5 Bazan JF, Bacon KB, Hardiman G, Wang W, Soo K, Rossi D, *et al.* A new class of membrane-bound chemokine with a CX3C motif. Nature 1997; 385:640–642.

6 McDermott DH, Fong AM, Yang Q, Sechler JM, Cupples LA, Merrell MN, *et al.* Chemokine receptor mutant CX3CR1-M280 has impaired adhesive function and correlates with protection from cardiovascular disease in humans. J Clin Invest 2003; 111:1241–1250.

7 Haskell CA, Cleary MD, Charo IF. Unique role of the chemokine domain of fractalkine in cell capture. Kinetics of receptor dissociation correlate with cell adhesion. J Biol Chem 2000; 275:34183–34189.

8 Kikuchi Y, Nonoguchi H, Machida K, Wakamatsu S, Koga H, Tomita K. Regulation of the apoptosis-related genes, Bax and Bcl-2, in the early stage of diabetes mellitus. Nephrology 2002; 7:294–302.

9 Kikuchi Y, Ikee R, Hemmi N, Hyodo N, Saigusa T, Namikoshi T, *et al.* Fractalkine and its receptor, CX3CR1, upregulation in streptozotocin- induced diabetic kidneys. Nephron Exp Nephrol 2004; 97:e17–e25.

10 Jerath M, Kwan M, Liu P. Chemokine receptors. In: Harrison JK, Lukacs NW, editors. *Atherosclerosis: the receptors, the chemokine receptors*. Totowa, NJ: Humana Press Inc.; 2007. pp. 199–233.

11 Umehara H, Bloom ET, Okazaki T, Nagano Y, Yoshie O, Imai T. Fractalkine in vascular biology: from basic research to clinical disease. Arterioscler Thromb Vasc Biol 2004; 24:34–40.

12 Lan NP. Atkins: macrophages in immune renal injury. In: Nielson EG, Couser WG, editors. *Immunologic renal disease*. Philadelphia: Lippincott Williams & Wilkins; 2001. p. 609.

13 Pan Y, Lloyd C, Zhou H, Dolich S, Deeds J, Gonzalo JA, *et al.* Neurotactin, a membrane-anchored chemokine upregulated in brain inflammation. Nature 1997; 387:611–617.

14 Kim M, Carman CV, Springer TA. Bidirectional transmembrane signaling by cytoplasmic domain separation in integrins. Science 2003; 301:1720–1725.

15 Nanki T, Imai T, Nagasaka K, Urasaki Y, Nonomura Y, Taniguchi K, *et al.* Migration of CX3CR1-positive T cells producing type 1 cytokines and cytotoxic molecules into the synovium of patients with rheumatoid arthritis. Arthritis Rheum 2002; 46:2878–2883.