Journal University of Kerbala , Vol. 16 No.4 Scientific . 2018

Role of chemokines (CCL2, CCL3, CCL4) in latent autoimmune diabetes of adult (LADA) and diabetes mellitus type2 (D.M.2)

دور الكيموكينات في السكري المناعي الذاتي المتأخر الحدوث للبالغين والسكري من النوع الثاني

Sawsan Mohammed Jabbar AL-Hasnawi*, **Suaad Mohammed Hassan Rasheed**/ Salman Aziz Adoos*****

***M.B.Ch.B., M.Sc., Ph.D. immunology, Dept. of immunology and microbiology /Karbala University College of Medicine.**

**** M.B.Ch.B., M.Sc., Ph.D. immunology /Kufa University College of Medicine.**

*****M.Sc., PhD. immunology, Dept. of immunology and microbiology /Kufa University.**

Abstract:

Immunological profile of LADA is not so clear, so detection of chemokines is quite important to determine the extent and direction of immune responses.

Objective**:** the aim of the study is to clarify the levels of chemokines in diabetes mellitus type 2 patients and LADA patients and show their relation with diabetes complications.

Methodology**:** level of the chemokines CCL2, CCL3and CCL4 were estimated in 90 subjects (34 known LADA cases, 36 D.M.2 cases & 20 healthy control participants), participating patients were recruited from Diabetes outpatient clinic in AL-Hussein Teaching Hospital in Karbala from June, 2013 through January, 2014. A clinical questioner containing personal data, family history, type of diabetes, hemoglobin A1C(HA1C), body mass index (BMI), diabetes duration& complications of diabetes was obtained from all patients. Statistical analysis done by using the statistical package for social sciences (SPSS) software for windows, data of all participants were entered and analyzed with appropriate statistical tests.

Results**:** significant high level of CCL2in LADA group compared to DM2, a positive correlation between CCL2 and duration of diabetes. Also CCL2 levels increased more with obese diabetic patients. .higher concentrations of CCL3 and CCL4 are associated with more complications in DM2 group.

Discussion**:** important role of CCL2 in development of autoimmune process in LADA as it is associated with increased levels among LADA patients. CCL2, CCL3 and CCL4 levels increased with patients having complications compared to those without complications.

Conclusion**:** chemokines are associated with complications of diabetes, in addition to the crucial role of CCL2 in development of autoimmunity of diabetes mellitus.

الخلاصة:

لا توجد معلومات او دراسات كافية حول الدور المناعي في تطورالسكري المناعي الذاتي المتأخر الحدوث للبالغين ،لهذا فان قياس مستوى الحركيات مهم جدا لفهم ذلك.

الهدف: الدراسة تهدف الى بيان مستويات الدم للكيموكينات لدى مرضى السكري من النوع الثاني والسكري المناعي الذاتي المتاحر الحدوث للبالغين واضهار العلاقة بين هذه الكيموكينات ومضاعفات السكري .

المنهجية: تم قياس، كل المرضى المشاركين تم تجميعهم من استشارية السكري في مشفى الحسين التعليمي في كربلاء من حزيران 2013 الى كانون الثاني من العام 2014. ولقد ضمنت المعلومات التالية في الاستفهام السريري لكل مريض:المعلومات الشخصية،التاريخ العائلي،نوع السكري،الهيموكلوبيين السكري،مؤشر كتلة الجسم،مدة المرض والمضاعفات الحاصلة بسبب السكري. النتائج حللت احصائيا باستخدام الحزمة الإحصائية للعلوم الاجتماعية لمايكروسوف ويندوز.
النتائج: ارتفاع مستوى ال سي سي ل 2 في الدم لدى مرضى السكري المناعي الذاتي المتاخر الحدوث للبالغين مقارنة مع مرضى السكري من النوع الثاني والفرق ذو دلالة احصائية كذلك مستوى ال سي سي ل 2 يزداد عند المرضى من فئة السمنة. ارتفاع تركيز ال سي سي ل 3 وال سي سي ل 4 يرتبط مع مضاعفات السكري من النوع الثاني.
المناقشة:الدور المهم لل سي سي ل 2 في تطور العملية المناعية لمرضى السكري المناعي الذاتي المتاخر الحدوث للبالغين لانه يزداد عند هؤلاء المرضى. مستويات ال سي سي ل2 وال سي سي ل 3 وال سي سي ل 4 في الدم تزداد عند المرضى المصابين بمضاعفات السكري مقارنة بغير المصابين بمضاعفات السكري .
الاستنتاج:الكيموكينات ترتبط مع مضاعفات السكري اضافة الى اهمية دور ال سي سي ل 2 في تطور العملية المناعية للسكري .

INTRODUCTION:

Diabetes mellitus is a group of metabolic disorders characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism occurred due to defects in insulin secretion, action, or both [1]. Diabetes mellitus can be classified into four types according to etiology; D.M.1, D.M.2, gestational diabetes and other specific types of diabetes. The majority of cases of diabetes fall into type 1 diabetes or type 2 diabetes [1].Type 2 diabetes is characterized by impaired β cell function and may be accompanied with changes of the immune system [2]. Latent autoimmune diabetes in adults (LADA) has some clinical features of type 2 diabetes & shows immunological abnormalities similar to those in type 1 diabetes, such as glutamic acid decarboxylase antibody (GADA) [3]. Insulin secretion was reported to be intermediate in LADA compared with type 1 and type 2 diabetes, whereas metabolic syndrome was similar in type 1 diabetes and LADA [4].

By definition chemokines are small chemoattractant glycopeptides. They have high homology in structure and were divided into 4 major classes; CXC, CX3C, CC and C, according to the number and position of the first two cysteine residues. Variant cells can produce chemokines after encountering an infection or agents that damage the tissue physically [5].

Interplay between chemokines and their receptors is important for migration of lymphocytes between blood, lymph nodes and tissues [6], and during an immune response the lymphocyte recruitment and activation is dependent upon the local chemokine production and the cellular expression of the appropriate receptor.

Different chemokines receptors could be expressed by CD4+ T cell subsets of T-cells; CCR5 and CXCR3 express by T-helper 1 cells , whereas T-helper 2 cells express CCR3 and CCR4 [7].

CCR5 ligands secretion such as CCL3, CCL4 and CCL5 was found to be related to T helper 1cells inflammatory responses, however secretion of ligands for CCR4, like CCL22, CCL2 and CCL17, associated with regulatory function of T helper 2 cells [8]. There are different chemokine receptors binds to many chemokine with strong affinity [9].

In type1diabetes a study has revealed elevation of CXCL10 (which is a chemokine with function to attract T cells and lead to more aggressive Th1-type response) [10]. Furthermore, other studies have shown a decrement in number of PBMC that express CCR5 and CXCR3 in patients with type1diabetes at early stages of disease in comparison to healthy controls subjects, suggesting that PBMC carrying chemokine receptor are re-localized to the inflamed pancreas [11].In type 1 diabetes infiltration of T-cells that express chemokine receptors are crucial in islet cell destruction [12].

The CC chemokine receptor 5 (CCR5) natural ligands are CCL3 (MIP-1alpha), CCL4 (MIP-1beta) and CCL5 which having relevant role in autoimmune diabetes. diabetes could be produced in the non-obese diabetic (NOD) mouse by transferring T-cell clones that secrete CCL3 and CCL5

which are of T-helper1 phenotype, whereas phenotype of T-helper 2 cells secreting CCL4 were unable to produce disease (the 2 phenotypes can lead to insulitis) [13].

In a study done in NOD mouse by Carvalho-Pinto leukocyte attraction by the CCR5 receptor was responsible for controlling development from insulitis to diabetes. The study revealed that treatment by neutralizing anti-CCR5 antibodies will lead to development of periinsulitis. So invasiveness and destruction of T-cells that infiltrated islet is governed by chemotaxis through CCR5 [14].

In stress or apoptosis of ß-cells, it can produce CCL3, CCL4 and CCL5, beside the secretion of the same chemokines by T-cells that infiltrate pancreas [14, 15]. In a study done on prediabetic individuals elevation of serum levels of CCL3 and CCL4 were found [16].Serum levels of CCL3 and CCL4 were elevated also in another study done on patients with newly diagnosed diabetes and low CCR5 expression [17]. Impairment of insulin signaling is known to be caused by CCL2 [18], so there is a relationship between CCL2 and insulin resistance [19].

Patients and method:

All patients who entered this cross-sectional study were selected randomly from Diabetes outpatient clinic in AL-Hussein Teaching Hospital in period from June 2013 through January 2014. The study population consisted of 90 individuals; the patients group aged from 30 to 73 years and with duration of disease between 1 month -25 years, 36 of whom have been diagnosed clinically as type 2 D.M., 34 patients was known LADA cases (having Glutamic acid decarboxylase antibody positive result) & 20 healthy control subjects. Serum samples were collected and stored at freezer (all samples allowed to be thawed only once) for chemokins analysis. Circulating chemokins concentrations of CCL2 (MCP-1), CCL3(MIP-1α) and CCL4(MIP-1β), were measured by ELISA technique using commercially available kits(Human ELISA Kit (eBioscience, USA),. Statistical method: by using the statistical package for social sciences (SPSS) software 2010, data of all participants (including HbA1c) were entered and analyzed with appropriate statistical tests. Descriptive statistics were presented as mean and standard deviation (SD) for the continuous variables and as frequencies and proportion for the categorical variables (No. and %). Analysis of variances (ANOVA) test was used to compare means of variables for three groups, and student's ***t*** test was used to compare means for two groups. Bivariate correlation was used to estimate the correlation between marker and other variable. Pearson correlation coefficient (R) value represented the strength of the correlation and the sign of R represented the direction of the correlation as followed: R value of < 0.4 indicated a weak correlation, $0.4 – 0.7$ indicated moderate correlations, and $R > 0.7$ indicated a strong correlation. The minus sign indicated an inverse (negative) correlation and the no-sign value indicated positive (direct) correlation. Level of significance (P-value) was set at ≤ 0.05 as a cutoff point for significant difference or correlation.

Note: Diagnosis of complications depends on patients records that are based on specialist diagnosis.

RESULTS:

LADA group had significantly higher values of CCL2 while D.M.2 had the least values. However, the difference was significant between D.M.2 vs. LADA (P=0.002), but it was not significant by comparing D.M.2 vs. control or LADA vs. control, see table 1.

Table (1): Comparison of mean values of Chemokines markers of the studied groups.

Marker	DM2 (No.=36)	LADA (No.=34)	Control (No.=20)	Compared groups	*P-value*
CCL2 (pg/ml)	91.9 ± 8.3	138.7 ± 53.9	115.3 ± 14.25	DM2 vs. LADA	**0.002** ***sig***
				DM2 vs. control	0.27 *NS*
				LADA vs. controls	0.28 *NS*
CCL3 (pg/ml)	9.1 ± 4.4	27.7 ± 9.7	4.5 – 2.5	DM2 vs. LADA	0.11 *NS*
				DM2 vs. control	0.91 *NS*
				LADA vs. controls	0.10 *NS*
CCL4 (pg/ml)	45.3 ± 8.12	31.35 ± 8.8	31.4 ± 4.15	DM2 vs. LADA	0.41 *NS*
				DM2 vs. control	0.52 *NS*
				LADA vs. controls	0.99 *NS*

* NS: not significant, sig : significant. p=0.05

In table (2) a positive significant correlation was found between CCL2 and duration of diabetes. Other markers showed mild to moderate correlation with variables (according to the value of R), although this correlation was statistically not significant.

Table (2): Correlation between circulating chemokines concentrations and variables for all patients groups combined

Variable	CCL2	p-value	CCL3	p-value	CCL4	p- value
HA1C	0.071	0.54	- 0.04	0.74	- 0.14	0.27
BMI	0.877	0.95	- 0.028	0.78	- 0.023	0.83
Duration of diabetes	0. 28	**0.018***	- 0.063	0.62	- 0.107	0.38
Age	- 0.006	0.95	0.12	0.26	0.14	0.19
Gender	- 0.11	0.30	- 0.027	0.80	- 0.049	0.68

* Correlation is significant at P<0.05, Hyphen-minus sign indicated inverse (negative) correlation.

In table 3, CCL2 levels are higher in obese categories DM2 and LADA patients. CCL3 and CCL4 levels increased with normal and overweight categories in both DM2 and LADA, although all result statistically non-significant**.**

Table (3): Comparison of mean values of markers according to BMI categories of DM2 & LADA cases. (Values represented the mean values of the markers)

BMI DM2	Normal (18 - 24.9)		Overweight (25 - 29.9)		Obese (>=30)		p-value
	Mean	SD	**Mean**	SD	**Mean**	SD	
CCL2(pg/ml)	105.0	9.0	81.1	12.3	110.9	12.0	0.28
CCL3(pg/ml)	56.0	7.5	68.0	15.8	60.4	1.1	0.76
CCL4(pg/ml)	62.5	26.4	47.5	11.6	44.0	8.3	0.77
BMI LADA							
CCL2(pg/ml)	146.9	16.1	124.1	12.0	173.4	25.4	0.16
CCL3(pg/ml)	108.5	91.5	101.3	26.8	58.5	25.5	0.76
CCL4(pg/ml)	52.3	23.1	41.5	15.8	19.5	4.4	0.42

*p=0.05

Table (4) shows that CCL2 levels associated with increased complications in D.M.2 group and it is more with diabetic foot.

Table (4): Comparison of mean values of markers according to complications in DM2 & LADA cases. (Values represented the mean values of the markers)

Marker for D.M.2	Complications					P-value
	Retinopathy	diabetic foot	IHD	CVA	Nephropathy	
CCL2(pg/ml)	86.8	142.0	81.0	114.0	8.0	0.39
CCL3(pg/ml)	56.0	None	None	62.0	None	0.26
CCL4(pg/ml)	101.0	20.0	84.0	51.0	13.0	0.84
Markers for LADA						
CCL2(pg/ml)	163.7	98.0	None	None	None	p>0.05
CCL3(pg/ml)	49.5	None				
CCL4 (pg/ml)	21.8	68.0				

* P. value is significant at <0.05 level.

CCL3 and 4 are associated with more complications in DM2, while CCL2 is associated with complications in LADA. See table (5).

Table (5): Comparison of the mean values of different markers according to the presence of complications among DM2 and LADA cases.

Markers DM2 cases	With complication No.=11		Without complication No.=25		P-value
	Mean	SD	Mean	SD	
CCL2(pg/ml)	**91**	16	**92**	10	0.96
CCL3(pg/ml)	**11**	7	**8**	5	0.79
CCL4(pg/ml)	**61**	22	**38**	7	0.97
LADA cases					
CCL2(pg/ml)	**154**	27	**135**	9	0.34
CCL3(pg/ml)	**14**	10	**31**	12	0.49
CCL4(pg/ml)	**28**	8	**32**	11	0.87

* p=0.05

DISCUSSION:

Current results show significantly higher values of CCL2 in LADA patients compared to D.M. type 2 patients (P=0.002). Values of CCL3 increased in LADA patients (27.7±9.7) compared to other groups (P- value>0.05).Various chemokines are associated with inflammatory autoimmunity like diabetes emphasizing there crucial role as a main goal for therapy development [20] .This result is in line with Pham study which found that LADA group had increased levels circulating CCL3 when compared to control and type 2 diabetic patients. It also reported an elevated level of CCL2 in LADA group compared to other groups but this result is statistically not significant and CCL4 level did not differ among studied groups [21].

No statistical difference found for HA1C, BMI, age, and gender with chemokines levels and this matches other studies [21].

CCL2 has a positive correlation with duration of diabetes p=0.018, this result means that CCL2 inflammatory effect appear clearly when duration of diabetes increased. A Persian study demonstrated that CCL2 correlates positively with the duration of disease in autoimmune diabetic patients [22].

Levels of CCL3 & CCL4 are higher in patients with complications compared to those without complications in D.M.2. CCL2 levels in LADA patients were higher in complicated cases. Also levels of CCL2, CCL3 are higher in patients with macrovascular complications (CVA, IHD) in D.M.2., this clarify the role of these inflammatory molecules on complications. These results are in line with Pham *et al.* [21] study. Zahra *et al.* [22] observed that the level of CCL2 chemokines is correlated with the diabetes complications. The CCL2 augmentation in autoimmune diabetic patients may also be a predictive marker for the possibility of patients progressing towards diabetes complications.

CONCLUSION:

1. Increased levels of CCL2 are associated with autoimmunity.
2. CCL2, CCL3 and CCL4 concentrations associated positively with complications of diabetes.
3. CCL2 could be used as a predictor for future development of diabetic complications.

REFERENCES:

1. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care, 2008; 31 Suppl 1: S55-60.
2. Shoelson SE, Lee J, Goldfine AB. Inflammation and insulin resistance. J Clin Invest, 2006; 116:1973–1801.
3. Leslie RD, Kolb H, Schloot NC et al. Diabetes classification: grey zones, sound and smoke: Action LADA 1. Diab Metab Res Rev, 2008; 24:511–519.
4. Hawa MI, Thivolet C, Mauricio D et al. Metabolic syndrome and autoimmune diabetes: Action LADA 3. Diabetes Care, 2009; 32:160–164.
5. Atkinson MA and Wilson SB. (2002). Fatal attraction: chemokines and type 1 diabetes.J Clin Invest; 110:1611-3.
6. Sallusto F, Mackay CR. (2004). Chemoattractants and their receptors in homeostasis and inflammation.Curr Opin Immunol; 16:724-31.
7. Von Andrian UH, Mackay CR. (2000). T-cell function and migration. Two sides of the same coin.N Engl J Med; 343:1020-34.
8. Antonelli A, Fallahi P, Ferrari SM, Pupilli C, d'Annunzio G, Lorini R and Vanelli M. Ferrannini E. (2008). Serum Th1 (CXCL10) and Th2 (CCL2) chemokine levels in children with newly diagnosed Type 1 diabetes: a longitudinal study. Diabet Med; 25:1349-53.
9. Tian Y, New DC, Yung LY, Allen RA, Slocombe PM, Twomey BM, Lee MM, Wong YH. (2004). Differential chemokine activation of CC chemokine receptor 1-regulated pathways: ligand selective activation of Galpha 14-coupled pathways.Eur J Immunol; 34:785-95.
10. Nicoletti F, Conget I, Di Mauro M, Di Marco R, Mazzarino MC, Bendtzen K, Messina A, Gomis R. (2002). Serum concentrations of the interferon-gamma-inducible chemokine IP-10/CXCL10 are augmented in both newly diagnosed Type I diabetes mellitus patients and subjects at risk of developing the disease.Diabetologia; 45:1107-10.
11. Hedman M, Faresjo M, Axelsson S, Ludvigsson J, Casas R. (2008). Impaired CD4 and CD8 T cell phenotype and reduced chemokine secretion in recent-onset type 1 diabetic children.Clin Exp Immunol; 153:360-8.
12. Atkinson M and Eisenbarth G. (2001). Type 1 diabetes: new perspectives on disease pathogenesis and treatment. Lancet; 358:221-229.
13. Bradley LM, Asensio VC, Schioetz LK, Harbertson J, Krahl T, Patstone G, Woolf N, Campbell IL, Sarvetnick N. (1999). Islet-specific Th1, but not Th2, cells secrete multiple chemokines and promote rapid induction of autoimmune diabetes. J.Immunol.162:2511-2520.
14. Carvalho-Pinto C, Garcia MI, Gomez L, Ballesteros A, Zaballos A, Flores JM, Mellado M, Rodriguez-Frade JM, Balomenos D, Martinez A. (2004). Leukocyte attraction through the CCR5 receptor controls progress from insulitis to diabetes in non-obese diabetic mice. Eur.J.Immunol.34:548-557.
15. Cnop M, Welsh N, Jonas JC, Jörns A, Lenzen S, Eizirik DL. (2005). Mechanisms of pancreatic beta-cell death in type 1 and type 2 diabetes: many differences, few similarities. Diabetes 54 (suppl2):S97–S107.
16. Hanifi-Moghaddam P, Kappler S, Seissler J, Muller-Scholze S, Martin S, Roep BO, Strassburger K, Kolb H, Schloot NC. (2006). Altered chemokine levels in individuals at risk of Type 1 diabetes mellitus. Diabet.Med.23:156-163.

17. Lohmann T, Laue S, Nietzschmann U, Kapellen TM, Lehmann I, Schroeder S, Paschke R, Kiess W. (2002). Reduced expression of Th1-associated chemokine receptors on peripheral blood lymphocytes at diagnosis of type 1 diabetes. Diabetes51:2474-2480.
18. Shi H, Tzameli I, Bjorbaek C, Flier JS. (2004). Suppressor of cytokine signaling 3 is a physiological regulator of adipocyte insulin signaling. J.Biol.Chem.279:34733-34740.
19. Herder C, Haastert B, Muller-Scholze S, Koenig W, Thorand B, Holle R, Wichmann HE, Scherbaum WA, Martin S, Kolb H. (2005). Association of systemic chemokine concentrations with impaired glucose tolerance and type 2 diabetes: results from the Cooperative Health Research in the Region of Augsburg Survey S4 (KORA S4). Diabetes54 Suppl 2:S11-S17.
20. Cameron, M. J., G. A. Arreaza, M. Grattan, C. Meagher, S. Sharif, M. D. Burdick, R. M. Strieter, D. N. Cook, and T. L. Delovitch. (2000). Differential expression of CC chemokines and the CCR5 receptor in the pancreas is associated with progression to type I diabetes.J. Immunol.165: 1102–1110.
21. Pham MN, Hawa MI, Roden M, Schernthaner G, Pozzilli P, Buzzetti R, et al. The Action LADA Study Group. Increased serum concentrations of adhesion molecules but not of chemokines in patients with type 2 diabetes compared with patients with type 1 diabetes and latent autoimmune diabetes in adult age: Action LADA 5.Diabet Med, 2012; 29:470–478.
22. Zahra Jamali , Mahmood Nazari , Hossein Khoramdelazad , Elham Hakimizadeh , Mehdi Mahmoodi , Mojgan Noroozi Karimabad ,Gholamhossein Hassanshahi , Mohsen Rezaeian , Parisa Balaei , SHokoofeh Darakhshan , Nahideh Masood Poor. (2013). Expression of CC Chemokines CCL2, CCL5, and CCL11 is associated with Duration of Disease and Complications in Type-1 Diabetes: A Study on Iranian Diabetic Patients. Clin. Lab.; 59, 11+12.