The Egyptian Rheumatologist (2013) **35**, 121–126

Egyptian Society for Joint Diseases and Arthritis

The Egyptian Rheumatologist

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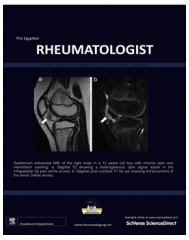

ORIGINAL ARTICLE

CXC ligand 13 in rheumatoid arthritis and its relation to secondary Sjögren's syndrome

Nahed Mounir Sherif, Mona Mahmoud Arafa, Soha Eldessouki Ibrahim *, Salwa Galal Moussa

Rheumatology and Rehabilitation Department, Faculty of Medicine – Ain Shams University, Cairo, Egypt

Received 23 January 2013; accepted 17 March 2013
Available online 20 April 2013

KEYWORDS

Rheumatoid arthritis;
Sjögren's syndrome;
CXCL13

Abstract *Aim of the work:* The aim of the present study was to measure the level of the chemokine CXC ligand 13 protein (CXCL13) in the plasma and unstimulated saliva of rheumatoid arthritis (RA) patients in order to find out its role in the disease activity and its relation to secondary Sjögren's syndrome (sSS).

Patients and methods: The study was conducted on thirty rheumatoid arthritis patients attending the Outpatient Clinic of Rheumatology and Rehabilitation department of Ain shams University Hospitals. The patients' group had been classified into *group* (*1*) which included fifteen RA patients associated with sSS diagnosed according to the American–European Consensus Group Classification Criteria and *group* (*2*) which included fifteen RA patients not associated with sSS. Ten healthy subjects were included as a control group. Patients were subjected to full history taking, clinical examination, and laboratory detection of CXCL13 level in the plasma and saliva of patients as well as the control groups using ELISA technique. Assessment of disease activity in RA patients was done using the disease activity score (DAS28).

Results: Plasma levels of CXCL13 were significantly higher in RA patients than control group ($p < 0.001$). Plasma levels of CXCL13 were significantly correlated with the RA disease activity ($r = 0.677$, $p < 0.001$) and disease duration ($r = 0.406$, $p < 0.05$), while the salivary levels were higher in those with sSS and correlated with sSS disease duration ($r = 0.536$, $p < 0.05$). A highly significant correlation was found between salivary CXCL13 and severity of sSS ($r = 0.816$, $p < 0.001$). Salivary levels of CXCL13 above 110 pg/ml may diagnose sSS with sensitivity 80% and specificity 84%.

* Corresponding author. Address: 31 Hassan Aflaton Street, Golf land, Cairo, Egypt.
E-mail address: soha_eldessouki@yahoo.com (S.E. Ibrahim).

Peer review under responsibility of Egyptian Society for Joint Diseases and Arthritis.

ELSEVIER Production and hosting by Elsevier

 http://dx.doi.org/10.1016/j.ejr.2013.03.004

Conclusion: The results of this preliminary study point out the importance of CXCL13 as a marker for RA disease activity, its role in diagnosing sSS, and estimation of sSS severity.

1. Introduction

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease characterized by an inflammatory erosive synovitis. This chronic inflammatory condition induces changes in the cellular composition and in the gene expression profile of the synovial membrane, resulting in intimal lining fibroblast like synoviocytes hyperplasia and sublining infiltration with mononuclear cells, especially CD4 + T cells, macrophages, and B cells [1]. The disease is characterized by acute painful inflammatory episodes, destructive changes of the joints that result in deformity and progressive functional impairment of joints [2]. Although RA is properly considered a disease of the joints, abnormal immune responses can cause a variety of extra-articular manifestations. In some cases, production of rheumatoid factor (RF) with the formation of immune complexes that fix complement contributes to extra-articular findings [3].

Secondary Sjögren's syndrome is usually included as an extra-articular manifestation of rheumatoid arthritis [4] which occurs in approximately 35% of patients, where the involvement of exocrine lacrimal and salivary glands happens. It was found that the average time between occurrence of their first symptoms and diagnosis of SS was 7.1 years [5].

Markers such as the erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) level have been incorporated into disease activity assessment in patients with RA [6]. However, both the ESR and CRP are nonspecific markers of inflammation and also can be increased by aging, anemia, and the presence of immunoglobulins, including RF [7]. Furthermore, normal levels of these markers are seen in some RA patients with active disease [8]. Therefore, these markers may not be helpful in all RA patients.

The chemokine CXC ligand 13 protein (CXCL13), also known as B-cell-attracting chemokine-1 or B-lymphocyte chemoattractant (BLC), is a CXC subtype member of the chemokine superfamily. Chemokines have been shown to orchestrate migration and preferential sequestration of B and T cells in inflammatory lesions [9].

BLC (CXCL13), a CXC chemokine, is the only chemokine which is known to specifically chemoattract B cells, belonging to both B-1 and B-2 subsets through the interaction with its receptor CXCR5. The gene for CXCL13 is located on human chromosome 4 in a cluster of other CXC chemokines. CXCL13 and CXCR5 together control the organization of B cells within follicles of lymphoid tissues and are expressed highly in the liver, spleen, lymph nodes, and gut of humans [10]. Plasma levels of CXCL13 provide an accurate test for defining the disease activity in RA patients [11]. Moreover, it was found that B cells have been implicated in the pathogenesis of SS and CXCL13 is a homeostatic chemokine that regulates B cell movement [12]. Therefore CXCL13 may be pathogenetically involved in the progression and severity of sSS.

A prior study on mice had approved the specific role of salivary CXCL13 in the diagnosis of sSS [13]. The current study may be the first one done on Egyptians using a measurement of CXCL13 levels in the saliva to determine its role in patients with sSS, as saliva can be obtained easily, providing a non-invasive, rapid and economical method.

The aim of this work is to measure the level of CXCL13 in the plasma and unstimulated saliva of RA patients in order to find out its role in the disease activity and its relation to secondary Sjögren's syndrome.

2. Patients and methods

The study was conducted on thirty rheumatoid arthritis patients attending the Outpatient Clinic of Rheumatology and Rehabilitation of Ain shams University Hospitals. Diagnosis was made according to American college of Rheumatology (ACR) and European League Against Rheumatism (EULAR) new classification criteria [14]. Ten healthy subjects were included as a control group. Informed consents were taken from each patient and control. The study was approved by Ain Shams medical ethical committee. The study was done in the period between June 2011 and April 2012.

The patients were classified into two equal subgroups as follows:

Group (*1*) which included fifteen RA patients associated with manifestations of sSS diagnosed according to the American–European Consensus Group Classification Criteria [15].

Group (*2*) which included fifteen RA patients not associated with manifestations of sSS.

Patients with malignant diseases, acute infection [16], on cyclophosphamide therapy [17], post head and neck radiation treatment, use of anti cholinergic drugs, facial paralysis, patients with sSS related to other rheumatological diseases were excluded from the study.

Patients were subjected to:

1. Full medical history taking,
2. Thorough clinical examination,
3. Routine laboratory investigations as:

- Complete blood count (CBC) using Coulter counter apparatus.
- Erythrocyte Sedimentation Rate (ESR) with Westergren method.
- C - reactive protein (CRP) in milligram per deciliter (mg/dl).
- Rheumatoid Factor (RF) with Latex fixation technique.
- Anti- cyclic citrullinated peptide assay (anti-CCP) by enzyme-linked immunosorbent assay (ELISA) (Euro-Diagnostica, the Netherlands).

4. Quantitative detection of CXCL13 level in plasma and saliva of the patients and control,
5. Using ELISA technique (Quantikine Human CXCL13 immuno-essay R & D systems, Minneapolis, MN: USA).
6. Assessment of disease activity in RA patients: Using the disease activity score (DAS28) [18]. Mild $\leqslant 3.2$, moderate $\geqslant 3.2$ but $\leqslant 5.1$, severe > 5.1. 6-Assessment of disease severity in sSS patients: using Lissamine green stain score [15]:

- The least severe condition is indicated by stains limited to the white of the eye between the lids toward the nose. This so-called nasal staining does not necessarily predict dry eye; it might be caused by environmental factors, such as pollution.
- The second level appears as stains in the white of the eye between the lids but toward the ear (Temporal conjunctiva) and called temporal staining.
- The third and most severe level occurs when the stain also appears on the cornea, indicating a break in the surface cells where bacteria can penetrate.

Statistical analysis: Analysis of data was performed by IBM computer using statistical package for social science (SPSS) version 16. Data were expressed as mean, standard deviation, and range. Number and percentage were used to describe qualitative variables. The comparison between two groups with parametric variables was done using independent sample *t*-test (*t*). ANOVA test was used to assess the statistical significance of the difference between more than two study group means. The correlation coefficient between two parametric parameters was calculated by using Pearson correlation coefficient. Chi-Square test was used to examine the relationship between two qualitative variables. The ROC Curve (receiver operating characteristic) provides a useful way to evaluate the Sensitivity and specificity for quantitative diagnostic measures that categorize cases into one of two groups. In all tests if ($p > 0.05$) it is non significant, if ($p < 0.05$) it is significant and if ($p < 0.001$) it is highly significant.

3. Results

The study was conducted on thirty RA patients and they were further classified according to whether or not they had of sSS into two equal subgroups (group 1 and group 2) respectively matching in age and sex, and ten normal subjects serving as control group.

3.1. The patients' groups

Group 1: It included 15 RA patients with manifestations of sSS (50%), they were 14 females (93.3%) and one male (6.7%), their age ranged from 30 to 62 years with a mean age of 50 ± 10.87. The disease duration of RA ranged from 2.5 to 30 years with a mean of 12.8 ± 9, the disease duration of sSS ranged from 1 to 6 years with a mean of 2.8 ± 1.6.

Group 2: It included 15 RA patients without manifestations of sSS (50%), they were 14 females (93.3%) and one male (6.7%), their age ranged from 17 to 62 years with a mean of age 43.53 ± 12.98. The disease duration of RA ranged from 0.2 to 14 years with a mean of 6.1 ± 4.9.

3.2. The control group

It included 10 normal subjects, 9 females (90%) and 1 male (10%), their age ranged from 27 to 60 years with a mean age of 41.9 ± 11.57.

On comparing the patients' and control groups, there was no statistically significant difference as regards age and sex distribution ($p > 0.05$).

4. Laboratory data

The laboratory data of the patients and controls are shown in Table 1.

Estimation of the plasma and salivary CXCL13 in all patients and controls is presented in Table 2.

5. Assessment of RA disease activity

- **Group 1**: DAS28 showed values ranging from 3.1 to 6.1 with a mean 5.5 ± 0.8. There was only 1 patient (6.7%) with mild RA disease activity, 8 patients (53.3%) had moderate RA activity, and 6 patients (40%) had severe activity of RA, as regards severity of

Table 1 Laboratory data of RA patients and control group.

Laboratory data	Rheumatoid arthritis (RA) patients				*p*	Control ($n = 10$)	
	With sSS ($n = 15$)		Without sSS ($n = 15$)				
	Range	Mean ± SD	Range	Mean ± SD		Range	Mean ± SD
ESR (mm/h)	30–130	82.5 ± 27.3	10–105	61.9 ± 23.5	0.04	8–11	8.4 ± 3.9
CRP (mg/dl)	3–64	29.1 ± 22.2	4–64	21.6 ± 19.2	0.33	4–7	6.8 ± 2
WBC count (10^3/mm^3)	2.5–16.6	7.7 ± 4.3	3.3–16.6	8.1 ± 4.1	0.07	4.1–11	8.1 ± 3.1
Hb concentration (gm/dl)	7.6–13.1	8.7 ± 3.4	7.1–14.1	9.2 ± 3.2	0.07	12–15.1	13.7 ± 2.6
RBC count (10^6/mm^3)	2.8–4.8	3.5 ± 0.7	2.5–4.8	3.9 ± 0.7	0.64	3.9–4.8	4.3 ± 0.8
Platelets count (10^3/mm^3)	130–470	260.5 ± 90.5	137–470	270.5 ± 92.5	0.36	235–440	330.5 ± 110.5
Lymphocytes count(10^3/mm^3)	0.9–2.9	1.7 ± 0.5	0.9–2.9	1.4 ± 0.7	0.45	1.5–3.3	2.1 ± 0.9
RF positive	No.	%	No.	%	0.07		
Anti-CCP	12	80	10	66.7			
	13	86.6	11	73.3			

RA: rheumatoid arthritis, sSS: secondary Sjogren's syndrome, No.: number, %: Percentage, ESR: erythrocyte sedimentation rate, CRP: C reactive protein, WBC: white blood cell, Hb: hemoglobin, RBC: red blood cell, RF: rheumatoid factor, Anti-CCP: anticyclic citrullinated peptide.

Table 2 CXCL13 plasma and salivary levels in the patients with and without sSS and controls.

Variables	Rheumatoid arthritis (RA) patients				p	Control (n = 10)	
	With sSS (n = 15)		Without sSS (n = 15)				
	Range	Mean ± SD	Range	Mean ± SD		Range	Mean ± SD
CXCL13 plasma (pg/ml)	30–250	136.7 ± 62.9	60–400	164.7 ± 90.7	0.335	9–15	11.5 ± 2.2
CXCL13 saliva (pg/ml)	85–355	206 ± 99.6	15–160	80 ± 42.1	0.001	8–13	10.6 ± 1.9

RA: rheumatoid arthritis, sSS: secondary Sjögren's syndrome.

sSS there were 4 patients (26.7%) with mild sSS, 5 patients (33.3%) had moderate sSS, and 6 patients (40%) had severe sSS.

- **Group 2**: DAS28 showed values ranged from 3.4 to 6.5 with a mean 5.1 ± 0.9. There were 3 patients (20%) with mild RA activity, 9 patients (60%) had moderate RA activity and 3 patients (20%) had severe active RA.

6. Comparison between the RA patients with sSS, RA without sSS and control group as regards plasma and salivary levels of CXCL13

There was a statistically highly significant difference ($p < 0.001$) between both patients' groups and the control group as regards the mean levels of plasma and salivary CXCL13.

7. The correlation between the disease duration of RA patients with sSS, RA without sSS and levels of plasma and salivary CXCL13

Concerning the RA disease duration, there was a statistically significant positive correlation with plasma CXCL13 levels ($r = 0.406$, $p < 0.05$), while there was no statistically significant correlation with salivary CXCL13 ($r = 0.049$, $p > 0.05$). Concerning the disease duration of sSS, there was a statistically significant positive correlation with salivary CXCL13 levels ($r = 0.536$, $p < 0.05$), while there was no statistically significant correlation with plasma CXCL13 levels ($r = 0.085$, $p > 0.05$).

There was no statistically significant correlation between the salivary levels of CXCL13 and each of ESR and CRP ($r = 0.242$, 0.21 respectively, $p > 0.05$), but there was a statistically significant positive correlation between plasma level of CXCL13 and ESR as well as CRP($r = 0.3$, $p < 0.05$).

CXCL13 plasma levels had a statistically highly significant positive correlation with the activity of RA (DAS28 ESR) ($r = 0.677$ & $p < 0.001$and no statistical significance with severity of SS ($r = 0.091$, $p > 0.05$). There was no statistically significant correlation between salivary CXCL13 and activity of RA ($r = 0.104$, $p > 0.05$), but a statistically highly significant positive correlation between salivary CXCL13 and severity of sSS ($r = 0.816$, $p < 0.001$).

8. ROC curve for diagnosis of sSS from salivary CXCL13 levels

The cut off value of salivary CXCL13 level to diagnose sSS is 110 pg/ml, with sensitivity 80% and specificity 84% (Fig. 1).

9. Discussion

The aim of this study was to measure the level of CXCL13 in the plasma and unstimulated saliva of RA patients in order to find out its role in the disease activity of RA patients and its relations to sSS.

Our study revealed a statistically highly significant difference in the plasma and salivary levels of CXCL13 in RA patients compared to controls ($p < 0.001$). Our results were in agreement with Rioja et al. [11] who found that there was a statistically highly significant difference in plasma CXCL13 levels between patients and normal controls ($p < 0.001$). Also it was in agreement with Kramer et al. [13], whose study was done on SS murine mice model, some had 1ry SS, others had sSS, and normal controls. Serum and saliva were collected from mice to detect CXCL13 levels, they found that there was a statistically highly significant difference between salivary CXCL13 levels between the diseased mice and healthy mice controls ($p < 0.001$). In our study the statistically highly significant difference between the salivary CXCL13 levels in patients without sSS (group2) and normal controls may indicate that some patients in this group were in the preclinical state of sSS, which needs further evaluation by finding out the relation between the CXCL13 salivary levels and the number of lymphocytes achieved by salivary gland tissue biopsies.

Rotondi et al. [12] found that B cells have been implicated in the pathogenesis of SS. CXCL13 is one of the homeostatic chemokines which are essential for lymphogenesis [19]. That is why it is secreted in normal individuals under basal circumstances [10]. So, it was not surprising to report a detectable amount of plasma and salivary CXCL13 in control subjects of our study.

On comparing the plasma levels of CXCL13 between patients with sSS (group 1) and those without sSS (group2), there was no statistically significant difference ($p > 0.05$), this could be explained by the high levels of circulating B cells which related to the pathogenesis of RA and SS disease as stated by Maini et al. [20]. While on comparing the salivary levels of CXCL13 between patients with sSS (group 1) and those without sSS (group2), there was a statistically highly significant increase in patients with sSS ($p < 0.001$), this was confirmed by the work done by Barone and his colleagues [21], who did their study on SS patients, some of them had SS secondary to RA, taking salivary glands biopsies to detect the expression levels of CXCL13 in salivary tissues in SS patients, and their results strongly suggested the active participation of CXCL13 in the development and maintenance of ectopic lymphoid structure in SS. As well as, it may reflect the immunological scenario in the salivary gland [22], but this result was not in agreement with what is stated by Gabriela et al. [23] who collected stim-

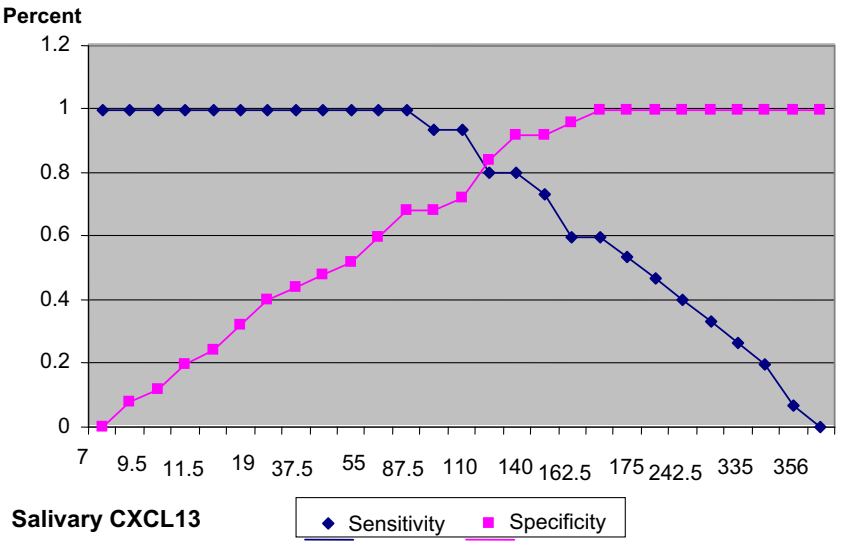

Figure 1 ROC curve for diagnosis of sSS from salivary CXCL13.

ulated saliva from patients with 1ry SS, patients with sSS, others with systemic autoimmune disease (SAD) without SS, and 32 healthy controls, to assess the saliva levels of CXCL13 in patients and healthy controls, they found that no significant difference between salivary levels of CXCL13 in sSS and SAD without SS, and this could be explained by the work done by Kramer et al. [13], who stated that, stimulated saliva samples from their mice patients may lead to a diluted CXCL13 concentration.

Our results also revealed that, there was a statistically highly significant correlation between the disease duration of RA and the plasma levels of CXCL13 ($r = 0.406$, $p < 0.001$). The levels of salivary CXCL13 showed a statistically highly significant correlation with the disease duration of sSS($r = 0.536$, $p < 0.001$).

In our work there was a statistically significant correlation between plasma levels of CXCL13 and CRP ($r = 0.366$, $p < 0.05$) as well as ESR ($r = 0.364$, $p < 0.05$), this was in accordance with the study done by Rioja et al. [11] who found a statistically highly significant correlation between CXCL13 plasma levels and ESR ($r > 0.5$, $p < 0.001$) as well as CRP ($r > 0.4$, $p < 0.001$).

Regarding the correlation between plasma CXCL13 and DAS 28, our results revealed a statistically highly significant correlation ($r = 0.677$, $p < 0.001$), Meanwhile Rioja et al. [11] showed a borderline significant correlation between DAS 28 and plasma CXCL13 levels, they also reported that plasma levels of CXCL13 provide an accurate test for defining the disease activity in RA patients, and this explained the above correlations. On the other hand, salivary levels CXCL13 in our study did not show any significant correlation with DAS 28 ($r = 0.104$, $p > 0.05$).

Concerning the severity of sSS, our study showed a statistically highly significant correlation between salivary levels of CXCL13 and severity of sSS ($r = 0.816$, $p < 0.001$), which was in accordance with what was stated by Barone et al. [21] who found that, the acquisition of lymphoid features by inflammatory foci in SS is critically associated with the enlargement of the inflammatory foci and with the expression of CXCL13. This also is in consistence with the findings of Kramer et al. [13], who did their study to detect salivary CXCL13 levels at early and late stage disease, correlating its levels with the severity and progression of SS; they found that salivary CXCL13 correlated with disease severity of sSS.

Salivary CXCL13 also had a statistically highly significant role in the diagnosis of sSS with a cut off value 110 pg/ml, where specificity was 84%, and sensitivity was 80%, this could be explained by the involvement of CXCL13 pathogenetically in Sjögren's syndrome development and progression, and its levels in the serum and saliva may reflect the severity of the SS disease in mice models as stated by Kramer et al. [13]. While correlation of sSS severity with plasma CXCL13 there were no statistical significant correlations ($r = 0.091$, $p > 0.05$), indicating that plasma CXCL13 might not be a severity marker in sSS, and if its plasma level is elevated in sSS it may be due to the activity of the original autoimmune disease [13].

Markers like rheumatoid factor (RF) and anti–cyclic citrullinated peptide antibodies, which help to define the autoimmune status in RA [24] are not very responsive to changes in levels of disease activity and are generally used only as prognostic markers of disease severity. The erythrocyte sedimentation rate (ESR), shown to be the component with the highest contribution in the DAS28 formula [25], is not very sensitive to short-term changes in disease activity and can be influenced by confounding factors, such as age, sex, fibrinogen levels, hypergammaglobulinemia, RF, and anemia [26]. Currently, C-reactive protein (CRP), an acute phase response protein, is the biomarker that is more widely used in clinical trials as a marker of the efficacy of potential disease-modifying compounds. In addition, studies have suggested that serum levels of (IL-6) correlate with levels of acute-phase proteins and may also be useful as a tool for monitoring disease activity in RA patients. However, due to the diurnal variability of IL-6 [27], and the lack of disease selectivity of CRP, there is a need for identifying and validating additional specific markers that can predict clinical outcomes.

In conclusion, our results showed that plasma CXCL13 is a novel biomarker of RA disease activity. The measurement of salivary CXCL13 could be a useful new method to make the definitive diagnosis of sSS, as it is correlated to severity of sSS in humans since the appearance of sSS in RA patients is independent of RA duration or activity [28]. It is considered a non-invasive technique and it possesses a smaller amount of proteins, meaning a minor risk of nonspecific interference and hydrostatic interactions, and because it is directly collected from the site of inflammation, it may reflect the immunological scenario in the salivary gland.

Conflict of interest

None.

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