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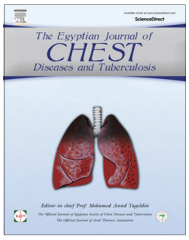

EDITORIAL

Study of the level of stem cell factor in patients with bronchial asthma

KEYWORDS

Bronchial asthma;
Stem cell factor;
Eosinophils

Abstract *Aim of the work:* The aim of this study was to assess serum level of stem cell factor in asthmatic patients and its relation to disease severity.
Methods: This case control study was carried out on 70 individuals classified into 20 healthy control subjects and 50 asthmatic patients that were admitted in Chest Department, Benha university hospital in the period between March 2013 and December 2014. Asthma diagnosis (history and PFT) and assessment of its severity were conducted according to the guidelines of Global Initiative for Asthma (GINA, 2011). Eosinophil percentage in the sputum of asthmatic patients was done. Measurement of stem cell factor (SCF) in the sera was done by Enzyme-Linked Immunosorbent Assay.
Results: Our results found a statistically significant difference (P value < 0.001) between asthmatic patients and control subjects in the mean values of SCF (1192.34 ± 789.89 versus 326.29 ± 274.38 respectively). There was a statistically significant difference (P value $= 0.001$) between eosinophilic and non eosinophilic phenotype (1457.77 ± 648.83 versus 130.59 ± 83.27 respectively). There was a statistically significant difference in the level of SCF among different degrees of asthma severity ($P = 0.001$). Also, there was a significant negative correlation ($r = -0.288$) between SCF levels and FEV1% and significant positive correlation between SCF levels and sputum ($r = 0.712$) and blood ($r = 0.548$) eosinophils% in asthmatic patients.
Conclusion: This study demonstrated that the serum level of SCF was higher in asthmatic patients especially among eosinophilic phenotype than among healthy control subjects. Also there was a significant association between higher SCF and higher levels of asthma severity, sputum and blood eosinophil%.

Introduction

Bronchial asthma is a common chronic disease of the respiratory system that affects approximately 300 million people worldwide. It is a heterogeneous disease, usually characterized by chronic airway inflammation. It is defined by the history of respiratory symptoms such as wheeze, shortness of breath, chest tightness and cough that vary over time and in intensity, together with variable expiratory airflow limitation [1]. Stem cell factor (SCF) is a dimeric molecule that exerts its biological functions by binding to and activating the receptor tyrosine kinase c-Kit. Activation of c-Kit leads to its autophosphorylation and initiation of signal transduction. Activation of c-Kit signaling has been found to mediate cell survival, migration, and proliferation depending on the cell type. Signaling from c-Kit is crucial for normal hematopoiesis, pigmentation, fertility, gut movement, and some aspects of the nervous system. Deregulated c-Kit kinase activity has been found in a number of pathological conditions, including cancer and allergy [2]. SCF can exist both as a transmembrane and a soluble protein. The soluble form of SCF contains a proteolytic cleavage site in exon 6. Cleavage at this site allows the extracellular portion of the protein to be released. The transmembrane form of SCF is formed by alternative splicing that excludes exon 6. Both forms of SCF bind to c-Kit and are biologically active [3]. Although SCF was initially described as a mast cell growth factor; it appeared to be a pleiotropic cytokine exerting its role

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at the first stages of bone marrow stem cell development, inducing eosinophil activation and basophil chemotaxis and survival [4]. SCF appears to play a significant role in eosinophil associated inflammation in allergic airway inflammation. SCF can play an important role in eosinophil activation, inducing degranulation, leukotriene, and chemokine production [5].

Aim of the work

This study was carried out to assess serum level of stem cell factor in asthmatic patients and to evaluate its relation to disease severity.

Subjects and method

This case control study was carried out on 70 individuals, 20 healthy control subjects and 50 asthmatic patients that were admitted in Chest Department, Benha University hospital in the period between March 2013 and December 2014. Subjects were divided into 2 groups: *Group I:* 50 patients with bronchial asthma and *Group II:* 20 apparently healthy individuals as a control group. *Group I* was further divided according to severity of asthma into: group Ia; mild persistent, group Ib; moderate persistent and group Ic; severe persistent asthma [6]. *Group I* was further divided into eosinophilic and non eosinophilic asthma according to percent of sputum eosinophil [7].

Inclusion criteria for group I

- Stable asthmatic patients with airway obstruction (FEV1/FVC < 70%) and with degree of reversibility in FEV1 of ⩾12% or 200 ml from pre bronchodilator value [6].
- Stopping corticosteroids 12 h before laboratory tests for severe persistent asthmatic patients.

Exclusion criteria

- Patients receiving high dose systemic corticosteroid (prednisone 1 mg/kg/day).
- Patients known to have systemic inflammatory diseases.

All subjects were subjected to the following:

I. Thorough history taking and clinical examination.
II. Plain chest X-ray (P-A view).
III. Pulmonary function test (spirometry pre and post bronchodilator) [8].
IV. Laboratory investigations:
1. Complete blood count.
2. Erythrocyte sedimentation rate.
3. Liver functions tests.
4. Kidney function tests.
5. Eosinophil percentage in the sputum of asthmatic patients. All patients gave sufficient sputum samples with no need for induced sputum technique. Sputum was treated by adding equivolume N-acetylcysteine, then it was spread on a slide then fixed with methanol 10%, the slide was stained with Leishman stain, then eosinophil percentage in the sample was calculated [9].
6. Measurement of serum level of stem cell factor by Enzyme-Linked Immunosorbent Assay (ELISA).

Principle of the Assay

SCF in standards and samples was sandwiched by the immobilized antibody and the biotinylated polyclonal antibody specific for SCF, which was recognized by a streptavidin–peroxidase conjugate. All unbound material was then washed away and a peroxidase enzyme substrate was added. The color development was stopped and the intensity of the color was measured [10].

Statistics

The data were entered into a personal computer. The statistics were performed using SPSS statistical program (version 20).

Results

Among the patients group, there were 43 (86%) females and 7 (14%) males. The ages of the patients ranged between 25 and 67 years (mean = 47.04 ± 12.08) and their BMI ranged from 19.53 to 47.61 kg/m^2. Among the control group, there were 15 (75%) females and 5 (25%) males. Their ages ranged between 36 and 68 years (mean = 45.15 ± 7.58). The patients and control groups were matched for sex, age and BMI ($P > 0.05$) (Table 1). Out of 50 asthmatic patients, 2 (4%) patients were mild persistent, 19 (38%) patients were moderate persistent and 29 (58%) patients were severely persistent (Table 2). And among asthma phenotype, 40 (80%) patients were eosinophilic and 10 (20%) patients were non eosinophilic (Table 3). There was a statistically significant difference (P value < 0.05) in the mean value of eosinophil percentage in sputum among asthma severity as it was higher among patients with severe asthma compared with the mild and moderate persistent. While percent of eosinophil in blood increased with increasing asthma severity with no statistically significant difference (Table 4). There was a highly statistical significant difference in the mean level of SCF between case and control group (1192.34 ± 789.89 versus 326.29 ± 274.38 respectively) (Table 5). There was a highly statistical significant difference in the level of SCF among different degrees of severity in asthmatic group as it was significant higher in severe persistent asthmatics compared with mild and moderate persistent (Table 6). There was a statistically significant difference in the level of SCF between eosinophilic and non-eosinophilic asthma (1457.77 ± 648.83 versus 130.59 ± 83.27 respectively) (Table 7). A significant positive correlation was found between SCF and eosinophil percent in sputum ($r = 0.71$) (Table 8 and Fig. 1) and blood ($r = 0.55$) (Table 8 and Fig. 2) as well as age ($r = 0.37$) (Table 8), also there was a significant negative correlation between SCF level and FEV1% predicted of asthmatic patients ($r = -0.29$) (Table 8 and Fig. 3).

Table 1 Demographic data and pulmonary function (spirometry) of studied groups.

	Cases	Control	*P* value
No.	50	20	
Age (years)	47.04 ± 12.08	45.15 ± 7.58	>0.05
Females no.	43 (86%)	15 (75%)	>0.05
Males no.	7 (14%)	5 (25%)	
BMI (kg/m^2)	30.38 ± 6.19	29.34 ± 3.13	>0.05
FVC (% pred.)	52.6 ± 17.65	88.45 ± 5.53	.000
FEV1 (% pred.)	63.64 ± 19.4	86.45 ± 5.59	.000
PEF (% pred.)	51.42 ± 19.62	82.7 ± 9.65	.000

Table 2 Classification of asthmatic patients according to severity.

Severity	Case 50 (100%)	
	No	%
Mild persistent	2	4
Moderate persistent	19	38
Severe persistent	29	58

Table 3 Classification of asthmatic patients according to phenotype.

Asthma phenotype	Case 50 (100%)	
	No	%
Eosinophilic	40	80
Non eosinophilic	10	20

Discussion

The results of this study revealed that there was a statistically significant difference between asthmatic patients and control subjects in the mean values of SCF (1192.34 ± 789.89 versus 326.29 ± 274.38 respectively). In addition there was a significant difference in the level of SCF among severity groups in asthmatic patients. Lei et al., had measured the serum concentrations of SCF and IL-31 in allergic asthmatic patients, which were significantly higher than those in normal control subjects [11]. This result was also in agreement with Makowska et al., who found that mean serum SCF level in the group of asthmatics was significantly higher as compared to healthy controls. The level of SCF was higher in patients with severe asthma as compared to patients with non-severe asthma [4]. Abd El Halim et al., assessed the level of SCF in peripheral blood in patients with asthma and its correlation with asthma severity and they found that there was a statistically significant difference (P value < 0.01) between asthmatic patients and control subjects in the mean values of SCF (1528.93 ± 465.40 versus 760.60 ± 427.62 respectively). Also there was a statistically significant correlation between severity of asthma and SCF levels (r = 0.526) [12]. Also, Safwat et al., found that mean serum SCF level in the group of asthmatics (n = 29) was significantly higher as compared to healthy controls and the level of SCF was higher in patients with severe asthma as compared to patients with non-severe asthma [13]. Other studies done by Kanabe et al., and Kim et al., showed increased production of SCF in different allergic diseases like asthma, allergic rhinitis and atopic dermatitis [14,15]. High level of SCF in bronchial asthma could be explained by Ashman (1999) who illustrated that SCF is a known inducer of mast cell proliferation and mast cell degranulation [16]. In previous studies done by Oliveira et al., they have identified that SCF production during allergic inflammation can contribute significantly to the induction of the eosinophilic inflammatory responses and the airway hyperreactivity via direct mast cell activation [5]. Also, Da Silva and Frossard, illustrated that stem cell factor (SCF) is a major mast cell growth factor, which could be involved in the local increase of mast cell number in the asthmatic airways. So, SCF expression increases in asthmatic patients [17]. Guntur and Reinero, had illustrated that stem cell factor (SCF), the ligand for c-Kit, is an important growth factor and activator of mast cells and eosinophils and that serum and airway SCF concentrations have been shown to correlate with asthma severity [18]. Our study showed that SCF was higher in eosinophilic asthma compared to non eosinophilic asthma (non eosinophilic asthma was defined according to Tsoumakidou et al., by presence of less than 2% eosinophil in sputum) [7]. This result can be explained by Hartman et al., who demonstrated that human peripheral blood eosinophils are a source of SCF and that result might contribute to a better understanding of the interactions between eosinophils and mast cells in allergic inflammation [19]. In a study made by Oliveira et al., they demonstrated that eosinophils have significant levels of surface c-Kit protein and SCF receptor. Also they demonstrated that SCF-activated

Table 4 Mean sputum and blood eosinophil among asthmatic patients.

	Mild persistent		Moderate persistent		Severe persistent		*P* value
	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	
%Sputum eosinophil	4–6	5 ± 1.41	0–10	5.47 ± 2.89	0–36	11.86 ± 8.91	⩽0.05
%Blood eosinophil	1.3–6	3.65 ± 3.32	1–8.6	3.99 ± 2.08	1–18.6	5.49 ± 4.23	>0.05

Table 5 SCF (pg/ml) among case and control groups.

	Case	Control	*P* value of *T*-test
Range	57–3219.6	68.7–1328.5	.000
Mean ± SD	1192.34 ± 789.89	326.29 ± 274.38	

Table 6 SCF value among different degrees of severity in asthmatic group.

	Severity						*P* value of *F*-test
SCF	*Mild*		*Moderate*		*Severe*		.001
	R	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	
	428 to 483	455.5 ± 38.89	82.3 to 1251.8	877.58 ± 330.02	57–3219.6	1449.37 ± 920.52	

Table 7 Comparison between different asthma phenotypes regarding SCF.

	Non eosinophilic	Eosinophilic	*P* value of *T*-test
Range	57–328.2	428–3219.6	.001
Mean ± SD	130.59 ± 83.27	1457.77 ± 648.83	

Table 8 Correlation between SCF values and different patients parameters.

	SCF	
Age	Pearson correlation	0.369
	Sig.	0.008
%Eosinophil in sputum	Pearson correlation	0.712
	Sig.	0.000
%Eosinophil in blood	Pearson correlation	0.548
	Sig.	0.000
FEV1% pred.	Pearson correlation	−0.288
	Sig.	0.04

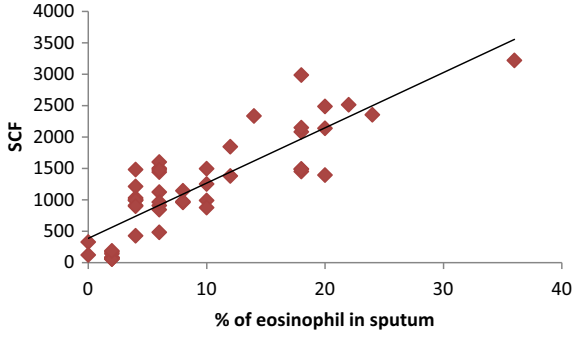

Figure 1 Correlation between SCF and eosinophil% in sputum.

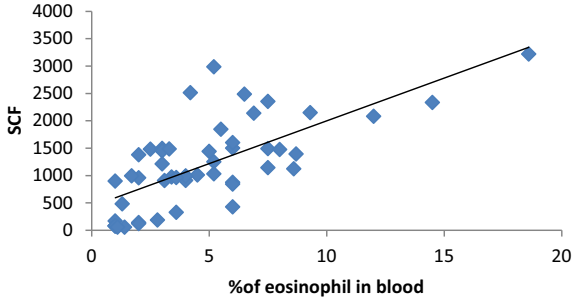

Figure 2 Correlation between SCF and eosinophil% in blood.

eosinophils degranulate and release eosinophil peroxidase and leukotriene C_4 in a dose-dependent manner [5]. Also Munitz and Levi-Schaffer, demonstrated that eosinophils synthesize, store, and release SCF [20]. The current study showed significant positive correlation between SCF levels and sputum ($r = 0.712$) and blood ($r = 0.548$) eosinophils% among asthmatic patients, higher sputum and blood eosinophils% were associated with higher SCF levels and vice versa, and a statistical significant negative correlation ($r = -0.288$) between SCF value and FEV1% predicted among asthmatic patients, lower FEV1% was associated with higher SCF levels and vice versa. These results also were in agreement with Abd El Halim et al., in which a statistically significant negative correlation ($r = -0.456$) was present between SCF levels and FEV1% among asthmatic patients. In addition, there was a significant positive correlation between SCF levels and blood ($r = 0.598$) and sputum ($r = 0.688$) eosinophils% in asthmatic patients [11]. Makowska et al., found that the level of SCF was higher in the group of patients with severe asthma as compared to

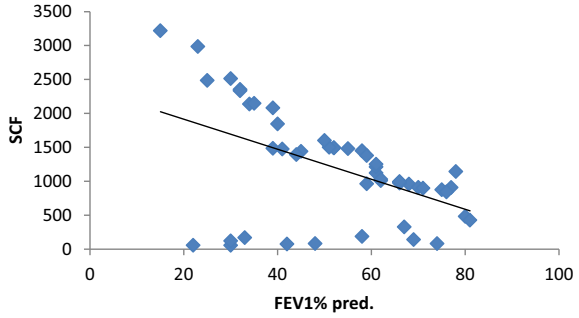

Figure 3 Correlation between SCF and FEV1% pred.

patients with non-severe asthma (1054 ± 41 pg/ml versus 819 ± 50 pg/ml; $P < 0.01$) [4].

In conclusion, this work demonstrated that serum level of SCF was higher among asthmatic patients than among healthy control subjects. Additionally, there was a statistically significant association between higher SCF levels and higher levels of severity of bronchial asthma and blood and sputum eosinophils percentage. Also SCF was higher among eosinophilic phenotype. These results suggest a role for SCF in asthmatic inflammation.

Conflict of interest

No conflict of interest.

References

[1] Global Initiative for Asthma (GINA), Global strategy for asthma management and prevention, 2015. Available from: <http://www.ginasthma.org/>.
[2] J. Lennartsson, L. Ronnstrand, Stem cell factor receptor/c-Kit: from basic science to clinical implications, Physiol. Rev. 92 (4) (2012) 1619–1649.
[3] K.E. Langley, L.G. Bennett, J. Wypych, S.A. Yancik, X.D. Liu, K.R. Westcott, Soluble stem cell factor in human serum, Blood 81 (3) (1993) 656–660.
[4] J.S. Makowska, M. Cieslak, M.L. Kowalski, Stem cell factor and its soluble receptor (c-kit) in serum of asthmatic patients-correlation with disease severity, BMC Pulm. Med. 9 (2009) 27.
[5] S.H. Oliveira, D.D. Taub, J. Nagel, R. Smith, C.M. Hogaboam, A. Berlin, N.W. Lukacs, Stem cell factor induces eosinophil activation and degranulation: mediator release and gene array analysis, Blood 100 (2002) 4291–4297.
[6] Global Initiative of Asthma (GINA), Asthma definition, 2011. Available at: <http://www.comginaasthma.comorg/upload/user/files/GINA.com>.
[7] M. Tsoumakidou, E. Papadopouli, N. Tzanakis, N.M. Siafakas, Airway inflammation and cellular stress innoneosinophilic atopic asthma, Chest 129 (5) (2006) 1194–1202.
[8] M.R. Miller, J. Hankinson, V. Brusasco, Standardisation of spirometry, Eur. Respir. J. 26 (2) (2005) 319–338.
[9] A.L.M. Palomino, M.H. Bussamara, B.M. Saraiva-Romanholo, M.A. Martins, M.T. Nunes, J.C. Rodrigues, Induced sputum in children and adolescents with asthma: safety, clinical applicability and inflammatory cells aspects in stable patients and during exacerbation, J. Pediatr. (Rio. J.) 81 (3) (2005) 216–224.
[10] K.E. Langley, L.G. Bennett, J. Wypych, S.A. Yancik, X.D. Liu, K.R. Westcott, K.M. Zsebo, Soluble stem cell factor in human serum, Blood 81 (3) (1993) 656–660.
[11] Z. Lei, G. Liu, Q. Huang, M. Lv, R. Zu, G.M. Zhang, Z.-H. Feng, B. Huang, SCF and IL-31 rather than IL-17 and BAFF are potential indicators in patients with allergic asthma, Allergy 63 (2008) 327–332.
[12] A. Abd El Halim, M. Abd El Gawad, S. Rabie, M. Kamal, Stem cell factor among asthmatic patients, Egypt J. Chest Tuberc. 60 (2011) 71–77.
[13] T. Safwat, N. Abdel Fattah, S. Abu-Hussein, Role of stem cell factor and its soluble receptor (c-kit) in diagnosis of asthmatic patients and patients with disease severity, Chest 140 (4) (2011), http://dx.doi.org/10.1378/chest.1117349.
[14] T. Kanabe, Y. Soma, Y. Kawa, M. Kashmida, M. Mizoguchi, Serum levels of soluble stem cell factor and soluble KIT are elevated in patients with atopic dermatitis and correlate with the disease severity, Br. J. Dermatol. 144 (2004) 1148–1153.
[15] Y.K. Kim, N. Nakagawa, k. Nakano, I. Sulakvelidze, J. Dolovich, J. Denburg, Stem cell factor in nasal polyposis and allergic rhinitis: increased expression by structural cells is suppressed by in vivo topical corticosteroids, J. Allergy Clin. Immunol. 100 (1997) 389–399.
[16] L.K. Ashman, The biology of stem cell factor and its receptor C-kit, Int. J. Biochem. Cell Biol. 31 (1999) 1037–1051.
[17] C.A. Da Silva, N. Frossard, Regulation of stem cell factor expression in inflammation and asthma, Mem. Inst. Oswaldo Cruz. 100 (1) (2005) 145–151.
[18] V.P. Guntur, C.R. Reinero, The potential use of tyrosine kinase inhibitors in severe asthma, Curr. Opin. Allergy Clin. Immunol. 12 (1) (2012) 68–75.
[19] M.L. Hartman, A.M. Piliponsky, V. VladislavTemkin, F. Levi-Schaffer, Human peripheral blood eosinophils express stem cell factor, Blood 97 (2001) 1086–1091.
[20] A. Munitz, F. Levi-Schaffer, Eosinophils: 'new' roles for 'old' cells, Allergy 59 (2004) 268–275.

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