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Evaluation Gene Expression of Stem Cell Factor and C-kit among Bronchial Asthmatic patients

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ABSTRACT
Background: Mast cells play an essential role in pathology of bronchial asthma. 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 when bind to its receptor (C-kit). Existing study aimed to evaluate role of gene expression of *SCF* and their receptor *C-kit* in development of bronchial asthma in Iraqi population.
Methods: present study included 100 patients diagnostic with bronchial asthma (59 females and 41 males) their ages range from 2 to 61 years and 30 healthy individuals (14 females and 16 males) with the same age range as control group DNA extracted from whole blood samples and then subjected to real time PCR for detection of gene expression.
Results: current data showed a significant correlation ($p<0.05$) between *SCF, C-kit* and asthma when high gene expression (mean of folding change) are appeared in asthmatic patients (5.20 and 4.03 respectively) compared to healthy control groups (1.00) . In present study, the highest gene expression of *SCF* and its receptor c-kit were appeared in sever persist asthma, moderate persist asthma and mild persist groups. Moreover, *SCF* and *C-kit* expression was influenced by treatment (3.54 and 3.63 respectively) in compared with untreated cases (3.78 and 4.97) ($p>0.05$) . The optimum cut-off values for gene expression of *SCF* and *C-kit* are ≥ 0.55, ≥2.328 respectively since there are associated with a perfect test for predicting asthma.
In conclusion: high gene expression of *SCF* and *C-kit* associated with increase severity stage of bronchial asthma without treatment and can be reduced by treatment.
Keyword: Asthma, *SCF, C-kit*, gene expression
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INTRODUCTION

Bronchial asthma is a chronic inflammatory and remodeling disorder of the airways, in which many cells, cellular elements, and cytokines play important roles [1, 2]. Mast cells from immune cells which have essential role in hypersensitivity reaction [3]. Stem cell factor (SCF), released by airway structural cells could play some role in increasing the number and/or activation of mast cells in the human airways [4]. Increased numbers of mast cells in airway smooth muscle linked to airway hyper-responsiveness. Mast cells release a large number of cytokines to change the airway environment and promote inflammation even though exposure to allergens is limited [5,6]. The stem cell factor, also called Kit ligand, steel factor or mast cell growth factor, is the ligand of the product encoded by the proto-oncogene c-*kit*. The gene for SCF in humans is located on chromosome 12q22-q24 and which is expressed in two forms, soluble (sSCF) and membrane bound (mSCF) after alternative splicing of the sixth exon, which encodes a proteolytic cleavage site [7]. Human mast cells produce more mSCF than sSCF while pulmonary fibroblasts and smooth muscle cells produce more sSCF than mSCF [7, 8]. SCF is involved in the early phases of hematopoiesis. In particular, this growth factor also acts as an important growth factor for human and murine mast cells, including *in vitro* proliferation and differentiation of immature CD34+ progenitors into mast cells in the bone marrow, and in peripheral blood. SCF initiates its effects by binding to the c-kit receptor, which results in receptor dimerization and activation of multiple signaling pathways; including the Erk1/2 and p38 mitogen-activated protein kinase pathways [7,9]. The C-Kit receptor (stem cell factor [SCF] receptor) is expressed on cells that respond to SCF, including the hematopoietic progenitor cells and lymphoid lines, melanocytes, germinal cells, eosinophil's in the peripheral blood, basophils and mast cells [10]. The stimulation of mast cells by SCF can induce their adhesion to extracellular matrix and degranulation, leading to the production and release of histamine, pro-inflammatory cytokines and chemokines. SCF also induces eosinophil adhesion and activation [11,12]. Current research aimed to evaluate role of gene expression of *SCF* and *C-kit* in development of bronchial asthma in Iraqi population.

MATERIALS AND METHODS

Study groups: The current study was conducted on 100 patients (59 females, 41 males).Patients were interviewed directly by using an anonymous questionnaire include; age, sex, the frequency of symptoms, drug, family history and smoking. The patients were diagnosed clinically by physician as having bronchial asthma. Diagnosis of bronchial asthma cases were carried out according to the treating physician and symptoms (i.e. wheeze, shortness of breath and cough) supported by evaluation of IgE and spirometry assessment before and after treatment with improving of FEV1 more than 12%.The control group consist of 30 apparently healthy individuals (16 male and 14 female) who had no pathological state at time of this study and without any history of systemic diseases , all of these individuals were matched to patients, in age group and gender,. In additional, present study was in agreement with ethics of Al-Zahraa Teaching Hospital Al-kut city,wasit,Iraq and verbal informed consent was obtained from all participants.

Classification of bronchial asthma: Depending on reading of spirometry staging according to the global initiative for asthma guidelines 2016 [13] and the National Asthma Education and Prevention Program classification [14], the cases of bronchial asthma of patients above 5 year are classified according to severity to four degrees including: intermittent, mild persist, moderate persist and sever persist whereas other cases with age ≤ 5 year are remain as non-classified.

Molecular study: Total RNA of all samples was extracted using the AccuZol kit and the protocol provided by the manufacturer**.** Total RNA was reversely transcribed to complementary DNA (cDNA) using (cDNA kit, СИНТОЛ Company / Russia). The procedure was carried out in a reaction volume of 25 µl according to the manufacturer's instructions. The expression levels of *SCF* and *C-kit* genes were estimated by Quantitative Real Time PCR (RT-qPCR). To confirm the expression of target gene, RT-PCR EVA Green assay was used. The mRNA levels of endogenous control gene β-Actin were amplified and used to normalize the mRNA levels of *SCF* and *C-kit* genes. Primers that used for RT-qPCR are listed in table (1).

Table 1. Primer Sequences Used in RT-qPCR

Gene	Primer	Sequence (5'→3' direction)	References
SCF	Forward	5-GAGCTCCAGAACAGCAAACG-3′	[7]
	Reverse	5′-CACTCCACAAGGTCATCCAC-3′	
C-kit	Forward	5′ - ATG AGA GGC GCTCGC GGC GC-3′	[25]
	Reverse	5′ - AGC TTG GCA GGA TCTCTA AC-3′	
β-actin (Reference gene)	Forward	5′ - CTGGAACGGTGAAGGTGACA-3′	[26]
	Reverse	5 – CGGCCACATTGTGAACTTTG-3′	

Statistical analysis: Statistical analyses were done using SPSS version 20 computer software (Statistical Package for Social Sciences) in association with Microsoft Excel 2010 and social science statistics. An estimate was considered statistically significant if its P value was less than an level of significance of 0.05. Gene expression was calculated using 2-ΔΔct method.

RESULTS

In present study most asthmatic cases have sever persist asthma (33.00%) followed by moderate persist asthma (23.00%), intermittent asthma (19%), mild persist asthma (15.00%) and non-classified asthma (10.00%)(figure 1). The current study, figure (2), show a high proportion of asthmatic patients with a positive familial asthma (61.00%) with a significant probability (p=0.030) in compared with patient without familial asthma (39.00%). Moreover, Figure (3) revealed that most patients in present study took treatment (66.00%).

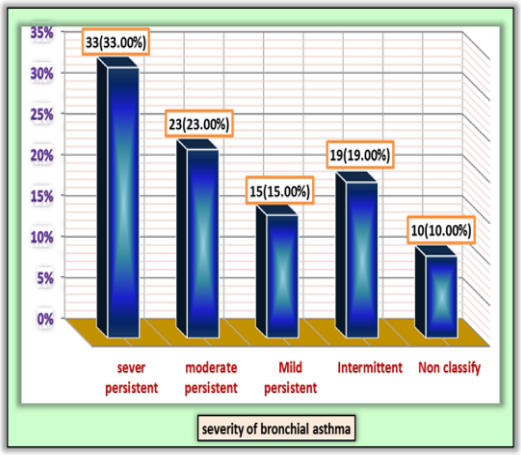

Figure 1. Distribution of asthmatic patients according to severity (X^2 = 0.607, p>0.05)

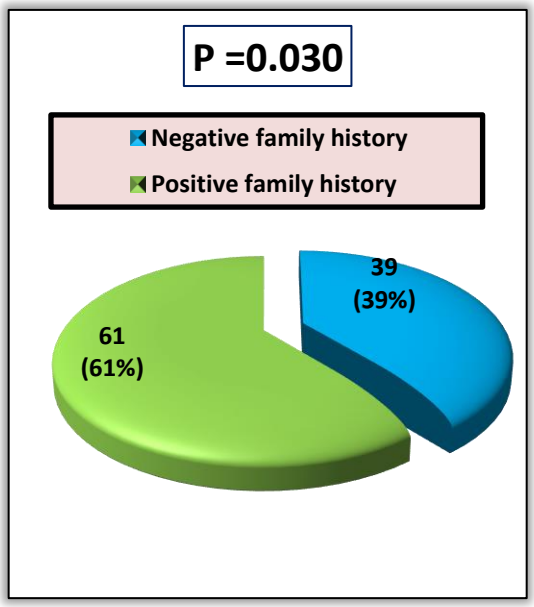

Figure 2. Distribution of asthmatic patients according to family history.

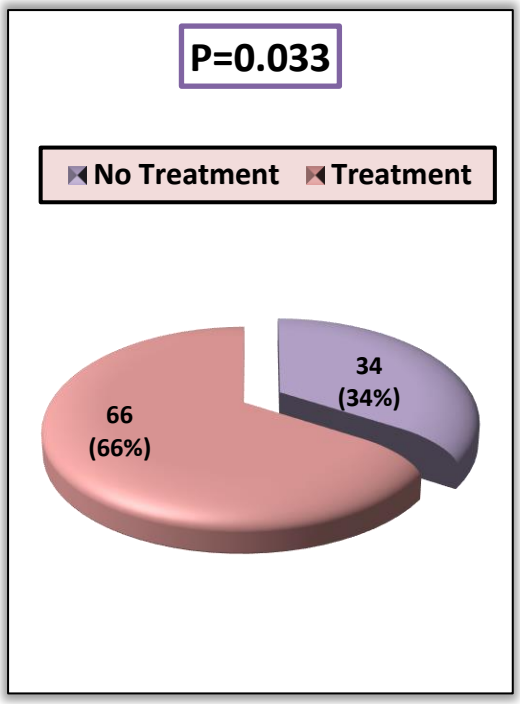

Figure 3. Distribution of asthmatic patients according treatment.

Analysis of relative gene expression was worked by using Real- Time Quantitative PCR and the 2 -ΔΔct Method of Livak et al., 2001 [15]. Table (2) and figure (4) showed a significant correlation ($p<0.05$) between SCF, C-kit and asthma when a high mean of folding change 2 -ΔΔct are appeared in asthmatic patients (5.205 and 4.03 respectively) compared to healthy control (1.00). the gene expression of C-kit effected by gender ($p>0.05$) as in figure (5A) and the noted folding changes of these genes are appeared in females (2.60 and 5.04 for SCF and C-kit respectively). Moreover, The highest gene expression of SCF and C-kit are looked in patients with familial asthma (2.88 and 7.64 respectively) compared to patients without family history (1.06 and 2.66 respectively) as in figure (5B).

According to tables (3) and figure (6), the highest gene expression of SCF was appeared in sever persist asthma followed by moderate persist asthma and mild persist group (11.43, 5.47 and 4.12 respectively). Moreover, the highest gene expression of c-kit was mainly located in sever persist followed by moderate persist asthma and mild persist asthma (5.09, 5.04 and 3.80 respectively). SCF and C-kit expression was influenced by treatment (3.54 and 3.63 respectively) in compared with untreated cases (3.78 and 4.97 respectively) as in table (4) and figure (7).

Figure (8) showed there are strong positive linear correlation between SCF and C-kit ($r= 0.7058$, $p= 0.0331$). The optimum cut-off values for 2-ΔΔct of SCF and C-kit gene expression, are ≥ 0.55 and ≥2.328 respectively since there are associated with a perfect test for predicting asthma. Gene expression of these cut-off values are 100% sensitive and specific, i .e. having a positive test can confirmed a possible diagnosis of asthma with 100% confidence. at the same time testing negative (up regulation of SCF, C-kit, are ≤ 0.55, ≤2.328, respectively) can exclude a possible diagnosis of asthma with 100% confidence.

Table 2. mRNA expression of *SCF* and *C-kit* in patients and control groups

Case-control	Mean CT gene	Mean CT actin	Mean ΔCT test	Mean ΔΔCT	Folding
SCF					
patients	14.80	11.23	3.57	-2.38	5.20*
Control group	16.28	10.33	5.95	0.00	1.00
C-kit					
patient	12.46	12.56	-0.1	-2.01	4.03*
Control group	14.48	12.57	1.91	0.00	1.00

*= Significant difference in compared with control group ($p<0.05$)

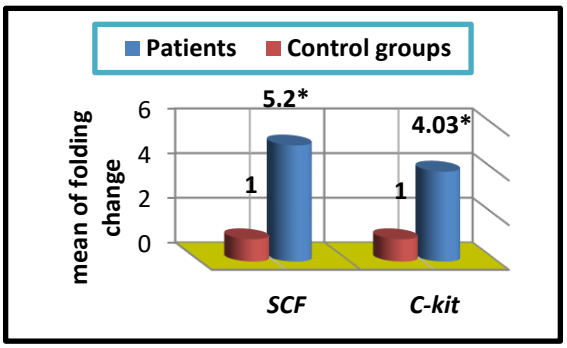

Figure 4. Compared mRNA expression of *SCF* and *C-kit* in patients with control groups (*= Significant difference in compared with control group ($p<0.05$))

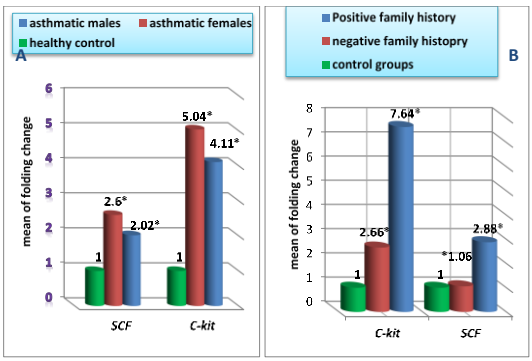

Figure 5. Distribution of mRNA, gene expression of *SCF, C-kit,* according to gender (A) and family

history (B). * = significant association in compared with control group (p<0.05).

Table 3. Distribution of mRNA, gene expression of SCF, C-kit in patients according to severity.

Case-control	Mean CT gene	Mean CT actin	Mean ΔCT test	Mean ΔCT control	Mean ΔΔCT	Folding
SCF						
Non classified	15.22	11.23	3.99	5.95	-1.96	3.89*
Intermittent	15.908	11.23	4.678	5.95	-1.272	2.41*
Mild Persist	15.1365	11.23	3.9065	5.95	-2.044	4.12*
moderate Persist	14.726	11.23	3.496	5.95	-2.454	5.37*
Sever Persist	13.664	11.23	2.434	5.95	-3.516	11.44*
Control group	16.28	10.33	5.95	5.95	0	1.00
C-kit						
Non classify	12.704	12.56	0.144	1.91	-1.776	3.40*
Intermittent	12.749	12.56	0.189	1.91	-1.721	3.29*
Mild Persist	12.543	12.56	-0.017	1.91	-1.927	3.80*
Moderate Persist	12.136	12.56	-0.424	1.91	-2.334	5.04*
Sever Persist	12.12	12.56	-0.44	1.91	-2.35	5.09*
Control	14.48	12.57	1.91	1.91	0	1.00

*= significant difference in compared with control (p<0.05).

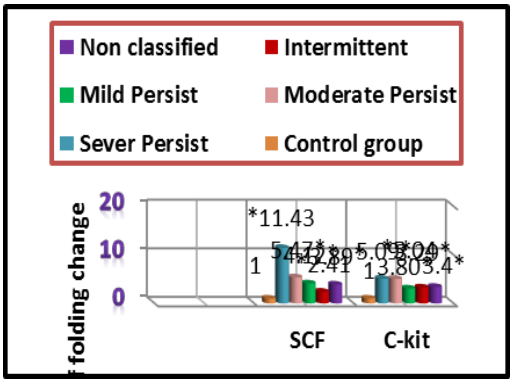

Figure 6. Distribution of mRNA, gene expression of SCF, C-kit according to severity (*= significant difference in compared with control (p<0.05)).

Table 4. mRNA expression of *SCF* and *C-kit* in treated and untreated patients.

Case-control group	Mean CT gene	Mean CT actin	Mean ΔCT test	Mean ΔCT control	Mean ΔΔCT	Folding
SCF						
Treated cases	15.354	11.23	4.124	5.95	-1.826	3.54

Untreated cases	15.259	11.23	4.029	5.95	-1.921	3.78
C-kit						
treated cases	12.61	12.56	0.05	1.91	-1.86	3.63*
Untreated cases	12.156	12.56	-0.404	1.91	-2.314	4.97

*= significant difference between treatment and gene expression (p<0.05).

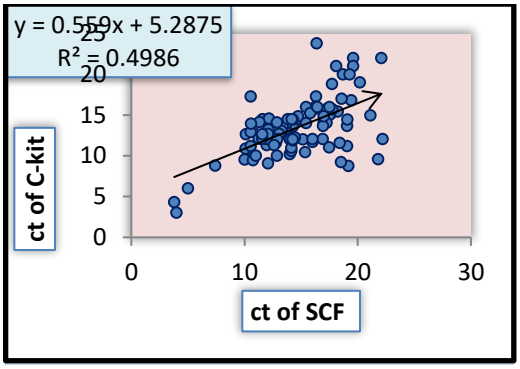

Figure 8.Linear correlation between *SCF* and *C-kit* (r=. 0.7058, p=0.0331)

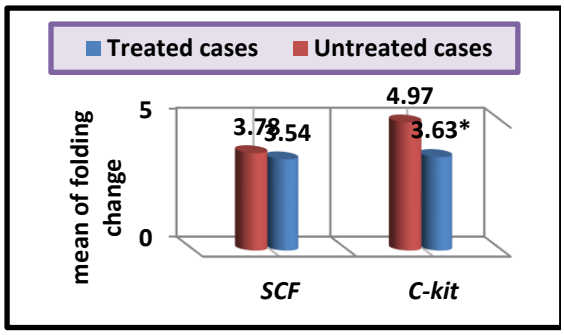

Figure 7. mRNA expression of *SCF* and *C-kit* in treated and untreated patients (*= significant difference in compared with untreated cases (p<0.05)).

Table 5.Validity parameters for the optimal cut-off value for selected quantitative indices when used as a test to diagnosis asthma differentiating it from healthy control

Positive if ≥ cut-off value	Sensitivity%	Specificity %	Accuracy%	PPV %	DP %
$2^{-\Delta\Delta ct}$ *SCF* ≥ 0.55	100.0	100.0	100.0	100.0	50.00
$2^{-\Delta\Delta ct}$ *C-Kit* ≥2.328	100.0	100.0	100.0	100.0	50.00

*PPV =Positive predictive value; DP= Disease prevalence

DISCUSSION

Present results showed increase mRNA expression of SCF and its receptor c-kit in asthmatic patients group when compared to control group and this observations are in line with Al-Muhsen et al.,]16[and Tayel et al.]7[who reported that, the expression of mRNA for SCF and its receptor c-kit were shown to be higher in the bronchi of patients with asthma as compared to controls group . The significance of a high level of SCF might be interpreted by the effect of SCF on mast cells when it act as chemo-attracting for progenitor mast cells from bone marrow to blood and this maintaining the process of asthma [17]. SCF is expressed in vitro by various cells from the airways, including the bronchial epithelial cells, lung fibroblasts, bronchial smooth muscle cells, endothelial cells, peripheral blood eosinophils and mast cells. Once released SCF acts on all cells that express the c-Kit receptor [4]. SCF initiates its effects by binding to the c-kit receptor, which results in receptor dimerization and activation of multiple signaling pathways; including the Erk1/2 and p38 mitogen-activated protein kinase pathways [18]. The Kit receptor is expressed on cells that respond to SCF, including the hematopoietic progenitor cells and lymphoid lines, melanocytes, germinal cells, eosinophil in the peripheral blood, basophils and mast cells [19].

Our study showed a clear relationship between family heredity and asthma pathogenicity and this result confirmed the role of the genetic factor in asthma. Also these results showed high gene expression of SCF and C-kit in patient with a family history of asthma, this the first study which evaluation gene expression of SCF and C-kit according to gender or family history of asthma.

In present study, the highest gene expression of SCF and its receptor c-kit was appeared in moderate persist asthma, sever persist asthma and non-classified group and this finding in agreement with Kowalski et al., [20] who demonstrated a pathophysiological link between SCF, its receptor c-kit, mast cells and severity of asthma. Da Silva et al., [21]. Also concluded that,

SCF and its receptor c-kit expression increases in the children and adult with sever or moderate asthma, and this is reversed after treatment with glucocorticoids.

As well as, the results revealed a significant negative correlation between SCF expression / c-kit and the treatment. This in agreement with Da Silva et al.,]4[and Makowska et al.,]22[who reported that, the higher expression of SCF and its receptor c-kit in asthmatic bronchi in comparison to healthy controls could be normalized by treatment with inhaled glucocorticosteroids. Glucocorticoids may reduce mast cells number and activation through decreased SCF expression [4,20,21]. Moreover, other study showed neutralizing SCF with a monoclonal antibody administered by inhalation during antigen provocation inhibits mast cell activation, bronchial inflammation [22]. Also Kim and his coworkers]24[suggested that inhibition of SCF signaling using c-Kit inhibitors such as masitinib might provide a novel therapeutic opportunity for the prevention of diabetes induced retinal vascular leakage [23].

Moreover, the increasing value of cut off for folding of SCF and C-kit, confirmed that these genes play an important role in pathology of asthma and is a reliable in diagnosing asthma. On other hand, only one study of Tayel et al.,]7[was found on this subject .Tayel and his colleagues [7]. determined that SCF expression at cut off point (0.52) is sufficient to discriminate asthmatic patients from control group and this findings in line with our study.

CONCLUSION

Present study showed a significant correlation between SCF, C-kit, and asthma infection when high gene expression are appeared in asthmatic patients. SCF and C-kit have a significant role in development of bronchial asthma and the highest expression of these genes mainly observed in females and in patients with familial asthma. Additionally, increase gene expression of SCF and C-kit are associated with increase severity stage of asthmatic patients which can be reduced by treatment.

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