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The Interfering Effects of PD-1 and TIPE2 in Bronchial Asthma

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ABSTRACT
Asthma is a chronic reversible airway disease, results from genetic – environmental interaction. Inflammatory processes may aggravate the severity of asthma and stimulate the airway remodeling. The aim of the current study is to evaluate the association between the negative inflammatory regulators; tumor necrosis factor α induced protein 8 like 2 (TIPE2) and Programmed cell death-1 (PD-1 with bronchial asthma in Iraqi population. blood samples are collected from 100 patients diagnosed with bronchial asthma (59 females and 41 males), their ages ranged between 2 to 61 years and 30 healthy individuals (14 females and 16 males) with the same age range. DNA extracted and then subjected to real time PCR for detection of gene expression. TIPE2 and PD-1 have low folding change of genes expression in asthmatic patients (0.01 and 0.10 respectively) compared to healthy control (1.00) which is reflect the significant differences ($p<0.05$). TIPE2 and PD-1 that have down expression (folding mean) in patients with familial asthma and the noted expression of these genes are clearly appeared in females (0.02 and 0.14 respectively). Also the lowest level of PD-1 gene expression was seen in unclassified group and then in moderate persist asthma (0.02, 0.11 respectively). While the lowest level of TIPE2 gene expression was seen in sever persist asthma, then moderate persist asthma and unclassified group (0.005, 0.006 and 0.008 respectively) .PD1 not affected by treatment but TIPE2 affected and increased. There are a significant negative correlation for each one of TIPE2, PD-1 with asthma infection.

Keywords: asthma, TIPE2, PD-1, gene expression

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INTRODUCTION

Asthma in humans constitutes a global health problem, affecting more than 300 million people of all ages [1]. Asthma is a polygenic, multifactorial disorder, which means that many factors contribute to its development. These factors are both genetic and environmental; accordingly, the combined action of several genes interacting as TIPE2 and PD-1 with one another and with environmental factors may be cause the clinical condition of asthma [2].TIPE2 is a newly identified immune negative regulator and mediates the maintenance of immune homeostasis [3]. It belongs to a member of tumor necrosis factor-a-induced protein-8 family which shares highly homologous sequence [4, 5]. TIPE2 is predominantly expressed on immune cells, such as lymphocytes and macrophages in mice. However, unlike murine TIPE2, human TIPE2 is also expressed on many kinds of non-immune cells, such as hepatocytes and neurons [6]. It has been reported that TIPE2 could negatively regulate both T cell receptor and Toll-like-receptor-mediated TIPE2 is known to negatively regulate inflammation, but the expression and significance of TIPE2 in asthma remain unclear[7]. In this study, we detected the expression level of TIPE2 in asthmatic patient with different age. In the same line, PD-1 also negatively regulate inflammation. The pathway PD-1/PD-L1 has been recognized to modulate and maintain peripheral CD4, including CD4+ Tregs and CD8+ T cell tolerance at several levels, in particular both T lymphocyte stability and integrity. More specifically, it can down-regulate self-reactive T cells during the presentation of self-antigen by dendritic cells (DCs) [8]. PD-1 is also able to directly promote interleukin-10 (IL-10) secretion by T cells and inhibit the maturation of DCs. PD-1 interaction shows a critical role also for the establishment of fetus tolerance [9,10,11]. The role of PD-1 and its ligands have already been observed in the regulation of autoimmune diseases [12,13,14] and in immunoregulatory functions in various microbial and infectious disease models [15-18]. These ligands were also reported to play a major role in tumor immunity [19, 20] and tissue transplantation [21, 22]. The relative contribution of the PD-1 to the development of allergic airway responses in bronchial asthma has recently been recognized [23]. This review will highlight recent findings on the potential role of this marker and TIPE2 in asthma.

MATERIALS AND METHODS

Study groups

The current study was conducted on 100 patients (59 females, 41 males). The patients were diagnosed clinically by physician as having bronchial asthma. Patients were interviewed directly by using an anonymous questionnaire include; age, sex, the frequency of symptoms, drug, family history and smoking. 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, all of these individuals were matched to patients, in age group and gender. Without any history of systemic diseases were clinically considered as control group in this study. In additional, present study was in agreement with ethics of Al- Zahraa Teaching Hospital and verbal informed consent was obtained from all participants.

Classification of bronchial asthma

Depending on clinical criteria and reading of pulmonary functional test, the cases of bronchial asthma of patient 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 under 6 year are unclassified.

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 PD1 and TIP2 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 PD1and TIP2 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
TIP2	Forward	5′- GGAACATCCAA GGCAAGACTG -3′	[42]
	Reverse	5′- AG CACCTCACTGCTTGTCTCATC -3′	
PD-1	Forward	5′- CCC TGG TGGTTG GTG TCG T- 3′	[43]
	Reverse	5′- GCC TGG CTC CTA TTG TCCCTC- 3′	
β-actin (Reference gene)	Forward	5′ -CTGGAACGGTGAAGGTGACA-3′	[44]
	Reverse	5′ -CGGCCACATTGTGAACTTTG-3′	

Statistical analysis: Data were translated into a computerized database structure. 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. Prevalence of variables as gender, ages and asthma severity are detected by approximate percent. When the data were normally distributed, an unpaired student's t test was used to compare the difference between patients with bronchial asthma and healthy controls. An estimate was considered statistically significant if its P value was less than an level of significance of 0.05.

RESULTS

Table (2) reveled there are no significant differences between patients and healthy individuals in the mean of age (29.1 ± 17.11 and 29.72 ±16.27 respectively) (p=0.977).The mean serum concentration of IgE significantly higher among cases with asthma (200.51ng/ml) compared to healthy controls (15.08ng/ml) (P<0.05). Also this results showed that most asthmatic patients are females (59%) but this not reflect significant differences between patients according to gender (p>0.05). The current study, figure (1), showed a high proportion of asthmatic patients with a positive familial asthma (61.00%) with a significant probability (p=0.030) in compared with patients without familial asthma (39.00%). Moreover, Figure (2) revealed that most patients in present study took treatment (66.00%).

Table 2.The patient-control comparison

patient-control Characters	Healthy controls	Asthmatic patients	P value
Age (years)			
Range	2-61	2-61	
Mean ± SD	29.1 ± 17.11	29.72 ±16.27	0.977
SE	3.177	1.635	
IgE(ng/ml)			
Range	24.329 – 419.398	2.447-38.293	
Mean	200.517	15.082	0.00001 *
SD	73.166	9.627	
SE	7.3166	1.758	

Gender	N	%	N	%	
Female	14	47	59	59	0.307
Male	16	53	41	41	0.312
Total	30	100	100	100	

*= Significant (p <0.05); SD= Standard Deviation; SE= Standard Error; N= Number

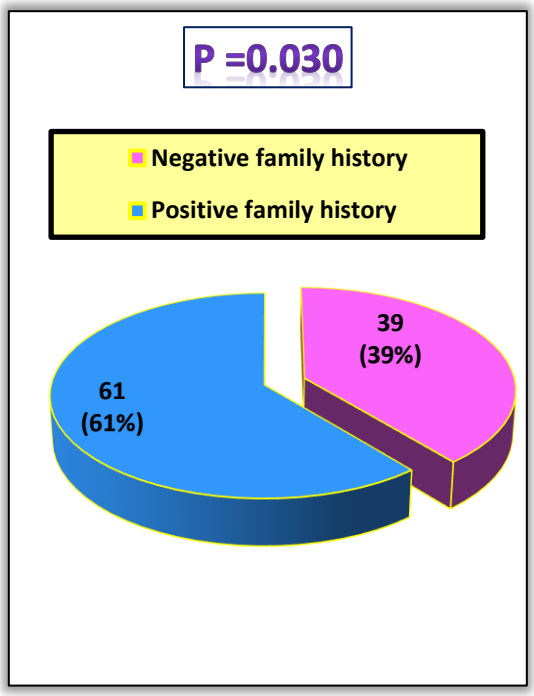

Figure 1. Distribution of asthmatic patients according to family history

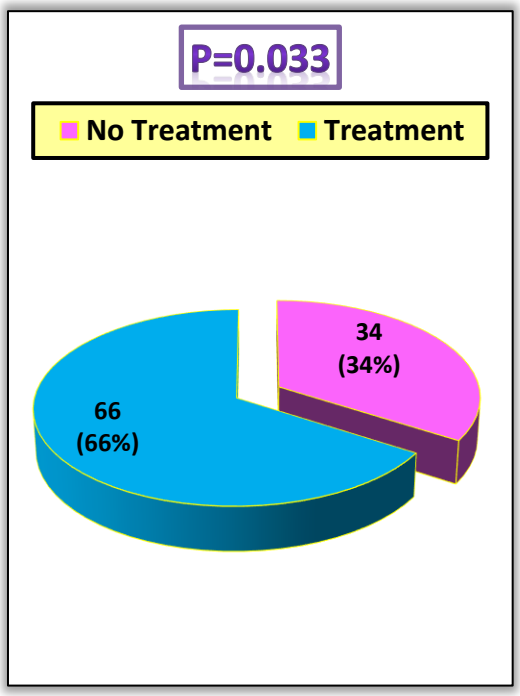

Figure 2. Distribution of asthmatic patients according treatment.

In table (3), Analysis of relative gene expression was worked by using Real- Time Quantitative PCR and the 2 -ΔΔct Method [41]. TIPE2 and PD-1 that have down expression (folding mean) in asthmatic patients (0.01 and 0.10 respectively) compared to healthy control (1.00) that reflect significant differences (p<0.05). In figure (3a) low mean folding of TIPE2 and PD-1 are demonstrated in patients with familial asthma (0.013 and 0.1 respectively) compared to patients without family history (0.014 and 0.23 respectively) and control (1.00).

Although the gene expression of TIPE2 and PD-1 influenced significantly by gender (p<0.05) as in figure (3b), the folding changes of these genes mainly appeared in females (0.02 and 0.14 respectively). According to negative correlation that appeared between TIPE2, PD-1 and asthma infection in above section, the lowest level of gene expression of TIPE2 was seen in sever persist asthma, moderate persist asthma and unclassified asthma (0.005, 0.006, 0.008 respectively) whereas gene expression of PD1 mainly decreased in unclassified asthma, sever persist asthma and moderate persist asthma (0.02, 0.11 and 0.12 respectively) as in table (4).

The gene expression of TIPE2 was affected by medication, when compared between treated cases (0.01) with not treated cases (0.005) and increased significantly (p<0.05), whereas PD-1 expression was not affected by treatment (0.15) when compared treated cases with not treated cases (0.19) as in table (4)

Table 3. mRNA expression of TIPE2 and PD-1 in asthmatic patients and control group .

Genes	Mean CT(gene)	Mean CT(actin)	Mean ΔCT(test)	Mean ΔΔCT	Mean Folding
	Mean	Mean	Mean	Mean	Mean
TIPE2					
Patients	19.40	20.49	-1.09	6.49	0.01*
Control	13.25	20.83	-7.58	0.00	1.00

PD-1					
Patients	19.34	16.74	2.6	3.22	0.10*
Control	16.42	17.04	-0.62	0.00	1.00

*= Significant difference (p<0.05)

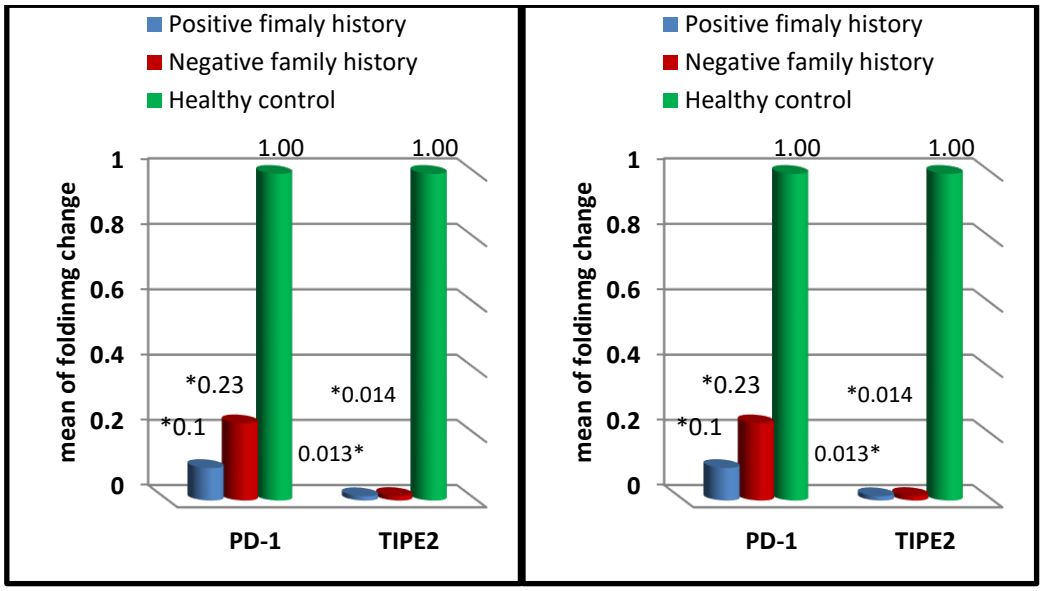

Figure 3. mRNA expression of *TIPE2* and *PD-1* in asthmatic patients according to family history (a) and gender (b). * = significant (p<0.05).

Table 4. The mRNA expression of TIPE2 and PD-1 according to severity and treatment of asthmatic patients and control group.

Genes and cases	Mean CT(gene)	Mean CT(actin)	Mean ΔCT(test)	Mean ΔCT(control)	Mean ΔΔCT	Mean Fold change ($2^{-\Delta\Delta CT}$)
TIPE2						
Unclassified patients	19.771	20.49	-0.719	-7.58	6.861	0.008*
Intermittent patients	18.569	20.49	-1.921	-7.58	5.659	0.019*
Mild persist patients	19.017	20.49	-1.473	-7.58	6.107	0.014*
Moderate persist patients	20.104	20.49	-0.386	-7.58	7.194	0.006*
Sever persist patients	20.441	20.49	-0.049	-7.58	7.531	0.005*
Control group	13.25	20.83	-7.58	-7.58	0.00	1.00
PD-1						
Unclassified patients	21.538	16.74	4.798	-0.62	5.418	0.02*
Intermittent patients	18.877	16.74	2.137	-0.62	2.757	0.14*
Mild persist patients	19.397	16.74	2.657	-0.62	3.277	0.13*
Moderate persist patients	19.246	16.74	2.506	-0.62	3.126	0.12*
Sever persist patients	19.125	16.74	2.385	-0.62	3.005	0.11*
Control group	16.42	17.04	-0.62	-0.62	0.00	1.00
TIPE2						
Treated patients	18.836	20.49	-1.654	-7.58	5.926	0.01*
Non treated patients	20.487	20.49	-0.003	-7.58	7.577	0.005
PD-1						
Treated patients	18.804	16.74	2.064	-0.62	2.684	0.15
Non treated patients	18.467	16.74	1.727	-0.62	2.347	0.19

*= significant correlation between severity and treatment and gene expression (p<0.05) in asthmatic patients.

DISCUSSION

Table (2) shows the mean age of patients in which 29.1 ± 17.11and 29.72 ±16.27 for control group (P = 0.977), this consistence with result of Mishra et al.,(2004) who found the mean age of asthmatic patients 30 ±11.2 and control group 29.23±12.94 (P > 0.05) but current results disagree with Vergara et al., (2010), who studied 429 non-related adult with asthma infection and 401 healthy individuals ,they reported that the mean age was 36.15± 18.32 and 34.98±17.8 years, respectively [23,24]. Gibson et al., (2010) and Nagasaki et al., (2013) assumed the prevalence of bronchial asthma in adults and older asthmatic patients may be due to inadequate treatment or healthcare of asthma, increase smoking or exposure to air pollutions and increase other pulmonary diseases or microbial infections in airways in some population (especially urban regions). In present study, most asthmatic patients are females (59%) and this finding agree with other studies [25,26,27]. Chhabra (2005) indicated that adult females were more infected by asthma, this effect may be because of increases of hormonal or biochemical differences related to sex, which may play a role in the pathophysiology of asthma. Other studies showed higher prevalence of asthma in adult women than men due to the sensitivity of female airway because of the representative female sex hormones which increase the secretion of cytokines and the total IgE level respectively [27]. How¬ever, progesterone inhibits histamine release from mast cells, and estrogen induces FoxP3+ regulatory T (Treg) cells [28]. This study also shows a significant correlation between serum level of IgE and bronchial asthma (P<0.001). Higher serum concentration of IgE in bronchial asthma due to its important role in the generation of mediators from mast cells that amplify the inflammatory response and in the development of bronchial hyper responsiveness [29,30].

Although PD-1 function is frequently associated with CD8+ T cell responses towards virus and cancer antigens, this results presented here as well as earlier studies underlined that PD-1 signals can limit pathological CD4+ T cell responses in asthma [31,32]. Moreover, earlier investigations demonstrated that PD-L1 blocking could lead to increased contact hypersensitivity reactions [33]. Regulatory function of PD-1 may be attributed to its repressive properties on T cells cytokine production or proliferation, as well as its stimulatory functions on development and activation of regulatory T cells [34]. Furthermore, Akbari et al.,(2010) reported a significant reduction in airway hypersensitivity as well as eosinophil numbers of broncho-alveolar lavage fluid in PD-1 murine models of asthma compared with wild type mice [35]. TIPE2 is a recently detected molecularly related with anti-inflammatory potential [36]. TIPE2 is an essential negative regulator of inflammation and immune homeostasis. TIPE2-deficent mice suffer from chronic inflammatory diseases and TIPE2-deficient T cells and macrophages produce significantly increased levels of inflammatory cytokines. [37]. According to present study, TIPE2 expression was significantly down-regulated in asthmatic patients compared to healthy control. This was in accordance with the results of previous studies, which identified the down-regulation of TIPE2 in PBMCs of patients with asthma [36,38]. Ma et al., 2013 that TIPE2 expression is negatively correlated with IgE, IL-4 and eosinophil counts in bronchial asthma [38].

In present result, the of TIPE2 and PD-1 are significantly affected by gender, this mean that genes play important role in development of asthma but most be refer to that female showed demand gene expression of TIPE2 and PD-1 that may be as causes of direct or indirect effects of hormones on gene expression. The current study showed a clear relationship between family heredity and asthmatic infection and this result confirmed the role of the genetic factors in asthma. Present results showed that gene expression of TIPE2 and PD-1 is high in patients with a family history of asthma, but unfortunately we not found a previous study which study the gene expression of TIPE2 and PD-1 according to gender and family history of asthmatic patients .

Our study showed that PD-1 and TIPE2 have negative regulation with severity of asthma. Dinga et al.,(2015) suggest that Crocetin may increase Foxp3 through TIPE2 to activate Treg to suppress the severity of asthma [37]. The current study is coming in line with study of Kalmarzi et al., (2017) who demonstrated a significant negative association between PD-1 and disease severity, symptom intensity, as well as, eosinophil counts in asthmatic patients [39]. Piconi et al. (2010) also reported that the percentage of IL-10 secreting, PD-1 expressing monocytes and B lymphocytes was significantly increased subsequent to sublingual immunotherapy in comparison with that of control group [34]. Furthermore, the PD-1 and TIPE2 may have an inhibitory role in asthma development and may be considered as a useful accessory immunotherapeutic approach for reduction of bronchial asthma [39,40]

CONCLUSIONS

TIPE2 and PD-1 have a negative significantly association with bronchial asthma and the lowest gene expression mainly seen in females and in patients with familial asthma. Moreover, decrease of TIPE2 and PD-1 gene expression are associated with increase severity of asthma.

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