

## Research Article

### TNF- $\alpha$ AS AN INFLAMMATORY CYTOKINE CAN NOT AFFECT PULMONARY FUNCTION IN ASTHMA PATIENTS

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#### ABSTRACT

The prevalence of asthma and other respiratory diseases has grown in the recent decades. In this study, we aimed to assess the relation between serum TNF- $\alpha$  as an inflammatory cytokine with some spirometry makers as respiratory function in a group of males with asthma. For this purpose twenty seven adult men with mild to moderate asthma were participated in this study by accessible sampling. Fasting serum TNF- $\alpha$  were measured and pulmonary function test (spirometry) was performed in order to measuring forced expiratory volume in 1 s (FEV1), forced vital capacity (FVC) and FEV1/FVC in each subject. Pearson's correlation coefficients were used to evaluate the correlations between - $\alpha$  concentration and all spirometry markers.  $P < 0.05$  was considered significant. No significant correlation was found in serum TNF- $\alpha$  with FEV1 ( $p = 0.87$ ,  $r = 0.032$ ), FVC ( $p = 0.61$ ,  $r = 0.10$ ) and FEV1/FVC ( $p = 0.35$ ,  $r = 0.19$ ) in studied subjects. In conclusion, our findings indicate that TNF- $\alpha$  as an inflammatory cytokine can not affect pulmonary function in asthma patients directly. Future studies will be needed to address the relative importance of inflammatory cytokines in airway inflammation.

**Keywords:** Airway Inflammation, Asthma, Spirometry

#### INTRODUCTION

Allergic diseases have increased in recent decades around the world. In developed countries, approximately 20-15% of public people are infected with allergic diseases. Chronic obstructive pulmonary disease and asthma are defined as chronic inflammatory disease relevant to respiratory pathways. There is evidence on systematic inflammation in their pathogenesis. One of the prominent features of this disease lies in hyper response of respiratory pathways, which causes narrowing of this respiratory tract in response to drugs or stimulant agents (Weiss *et al.*, 2004). On the other hand, inflammatory processes in asthma are affected by a complex network of cytokines and growth factors, which are not only secreted by inflammatory cells but also by other tissues such as epithelial cells, fibroblasts and smooth muscle cells (Settin *et al.*, 2008). Inflammation of the mucosa in respiratory pathways is associated with acute or chronic inflammation in asthmatic patients (Bousquet *et al.*, 2000). TNF- $\alpha$  is an inflammatory cytokine secreted by several proinflammatory cells such as macrophages and mast cells. It is introduced as the cause of inflammatory responses and regulation of the immune system (Aggarwal *et al.*, 2003). Disruption in level of TNF- $\alpha$  or lack of regulatory responses to TNF- $\alpha$  was reported in several inflammatory diseases. Several studies showed that increased secretion of TNF- $\alpha$  is associated with increased clinical asthmatic symptoms and inflammation of respiratory pathways (Boulet *et al.*, 2005).

Based on this evidence, it can be concluded that TNF- $\alpha$  affects the relationship between mast cells and smooth muscles, which is associated with increased hyper response of respiratory pathways. It is also possible that TNF- $\alpha$  is effective in the absence of a systematic inflammatory response in the respiratory pathways in asthmatic patients. In this regard, higher levels, increased levels, or increased expression of this inflammatory protein is reported in respiratory pathways of asthmatic patients (Ying *et al.*, 1991; Bradding *et al.*, 1994).

However, it is not clear whether disruption in TNF- $\alpha$  level leads directly or indirectly to inflammation of the respiratory pathways, it is strongly affected by this disease, or it indirectly leads to increased

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resistance to respiratory pathways. Hence, this study aimed to determine the relationship between serum levels of TNF- $\alpha$  with spirometric parameters such as forced expiratory volume in 1 S (FEV1), forced vital capacity (FVC), FEV1 / FVC, indicating the severity of asthma, in a group of patients with mild to moderate asthma.

## **MATERIALS AND METHODS**

### ***Subjects and Experimental Design***

In present study, to determine the relation between serum TNF- $\alpha$  as an inflammatory cytokine with spirometry markers in asthma patients, twenty seven adult males aged  $38 \pm 6$  old and height  $173 \pm 2$  cm with mild to moderate asthma were participated in this study by accessible sampling. Asthma diagnosis and its severity were determined by FEV1/FVC. Written consent was obtained from each subject after the experimental procedures and possible risks and benefits were clearly explained. Inclusion criteria to study were asthma history of At least for 3 years ago. Participants were non-athletes, non-smokers and non-alcoholics. The exclusion criteria were infections, renal diseases, hepatic disorders, diabetes and cardiovascular disease and other chronic diseases.

### ***Anthropometric, Pulmonary Test and Blood Analysis***

All anthropometric measurements were made by the same trained general physician. Weight and height were measured in the morning, in fasting condition, standing, wearing light clothing and no shoes. Body mass index was calculated as body mass (in kilograms) divided by height squared (in square meters). Abdominal circumference and hip circumference were measured in the most condensed part using a non-elastic cloth meter. Waist-to-hip ratio was calculated as abdominal circumference divided by hip circumference.

Subjects were asked to refrain from tea, coffee, chocolates and caffeinated soft-drinks on the day of recording Spirometry (Minispire, Italia). Subjects were instructed to take maximum inspiration and blow into the pre-vent pneumotach as rapidly, forcefully and completely as possible for a minimum of 6 seconds, followed by full and rapid inspiration to complete the flow volume loop. The best of the three trials was considered for data analysis. Spirometry test used to assess FEV1, FVC, FEV1/FVC% and other respiratory function parameters.

All participants refrained from any severe physical activity 48 h before measurements. Blood samples were collected after an overnight fast between 8:00 a.m. and 9:00 a.m. in order to measuring serum TNF- $\alpha$  by Eliza method. Blood samples were dispensed into EDTA-coated tubes and centrifuged for 10 minutes in order to separate serum. TNF- $\alpha$  was determined by ELISA method (Enzyme-linked Immunosorbent Assay for quantitative detection of human TNF- $\alpha$ , Austria) and the intra- assay and inter-assay coefficient of variation of the method were 6.0 and 7.4 respectively.

### ***Data Analysis***

Data were analyzed by computer using the Statistical Package for Social Sciences (SPSS) for Windows, version 15. Kolmogorov-Smirnov test was used to determine of normal status of the data. Pearson correlations were used to establish the relationship between TNF- $\alpha$  concentration and all spirometry markers. A p-value of less than 0.05 was considered to be statistically significant.

## **RESULTS**

As previous mentioned, this study was aimed to determine serum TNF- $\alpha$  in relation to some spirometry markers in asthma patients. Tables 1 summarize anthropometrical markers of studied patients. The data were reported as mean and standard deviation. Mean and SD of spirometry markers and TNF- $\alpha$  were also summarized in table 2. Data of Pearson correlation coefficients showed no significant correlation between serum TNF- $\alpha$  and FEV1 in studied patients ( $p = 0.87$ ,  $r = 0.032$ , Figure 1). Serum TNF- $\alpha$  was not correlated with FVC in subjects ( $p = 0.61$ ,  $r = 0.10$ , Figure2). No other significant correlations were found between TNF- $\alpha$  with other spirometry markers such as FEV1/FVC ( $p = 0.35$ ,  $r = 0.19$ , Figure 2).

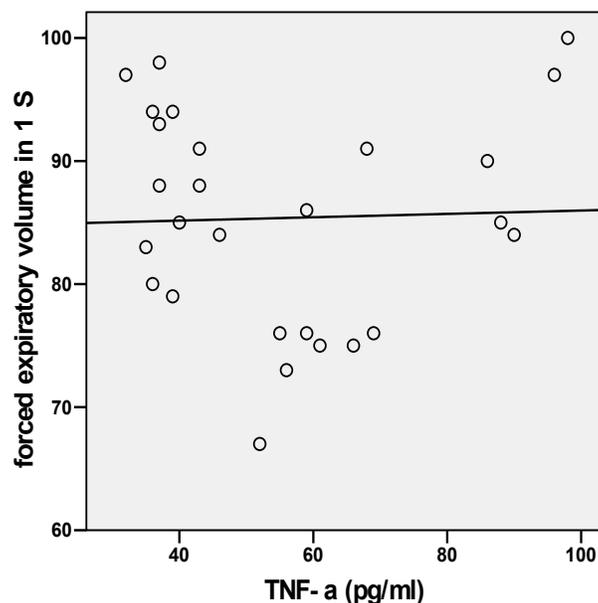
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**Table 1: Descriptive statistics of anthropometrical markers in studied patients**

	N	Minimum	Maximum	Mean	Std. Deviation
Age (year)	27	26	50	38.15	6.449
Height (cm)	27	170	177	173.41	2.135
Weight (kg)	27	77	114	92.85	10.995
Abdominal (cm)	27	89	130	104.07	10.092
Hip (cm)	27	91	128	104.37	8.266
WHO	27	.94	1.10	.9970	.03851
BMI (kg/m2)	27	26	38	30.81	3.541

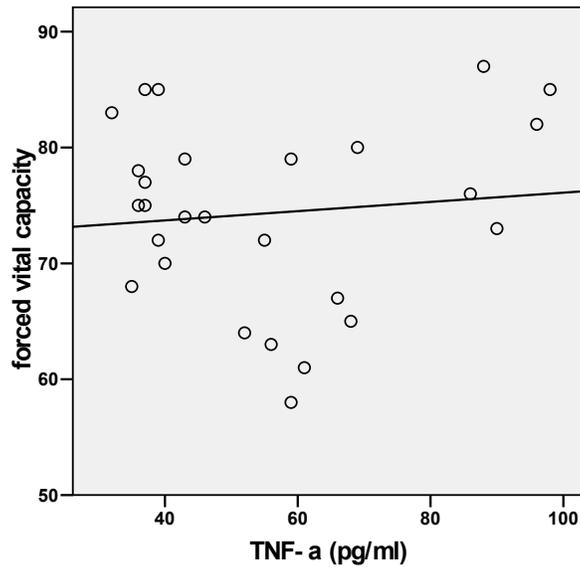
**Table 2: Descriptive statistics of Spirometry markers and serum TNF- $\alpha$  in studied patients**

	N	Minimum	Maximum	Mean	Std. Deviation
forced expiratory volume in 1 s	27	67	100	85.37	8.767
forced vital capacity	27	58	87	74.33	7.913
FEV1 / FVC	27	63	75	68.93	3.257
Peak expiratory flow	27	56	99	78.04	14.743
FEF %25-%75	27	32	77	59.44	14.716
FEF %75	27	28	80	53.56	15.749
Expiratory vital capacity	27	73	100	89.56	7.587
TNF-a (pg/ml)	27	32	98	55.67	20.649

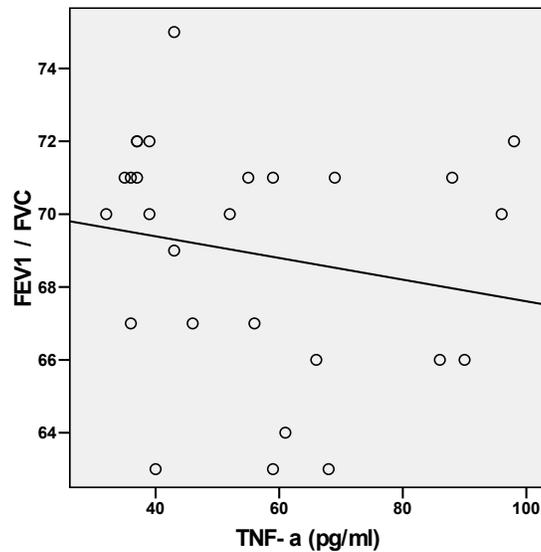


**Figure 1: No significant correlation between serum TNF- $\alpha$  and FEV1 in studied patients**

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**Figure 2: No significant correlation between serum TNF- $\alpha$  and FVC in studied patients**



**Figure 3: No significant correlation between serum TNF- $\alpha$  and FEV1/FVC in studied patients**

**DISCUSSION**

Several studies reported that asthma is an inflammatory disease associated with systemic inflammation and inflammation of the respiratory pathways (Eizadi *et al.*, 2011; Bousquet *et al.*, 2000; Kony *et al.*, 2004). Earlier studies cited disruption in inflammatory and anti-inflammatory cytokine levels in the presence of this disease (Kim *et al.*, 2008; Castro-Rodríguez *et al.*, 2007; Butland *et al.*, 2008). This is because most studies attributed severity of asthma to increased inflammatory cytokines or decreased anti-inflammatory cytokines (Yokoyama *et al.*, 1997; Bradding *et al.*, 1994). However, the major mechanisms responsible for disruption in the cytokines secreted from adipose tissue or other body tissues leading to prevalence or severity of asthma are not fully specified. For example, although several previous studies supported increased levels of TNF- $\alpha$  in asthmatic patients compared to healthy subjects (Godding *et al.*, 1995), these studies did not justify how this inflammatory cytokine affects these patients. In the present study, no significant correlation was observed between serum levels of TNF- $\alpha$  with each one of the

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spirometric parameters (FVC, FEV1, FEV1 / FVC) in asthmatic patients. Based on the findings obtained in this study, changes in the levels of inflammatory cytokine leading to changes in respiratory function parameters in asthmatic patients do not follow a specific pattern. Nevertheless, many previous studies have emphasized that TNF- $\alpha$  is a cytokine or mediator of mast cells, which is effective in hyper response of respiratory pathways (Kips *et al.*, 1992; Thomas *et al.*, 1995). TNF- $\alpha$  accelerates release of histamine from human mast cells directly and participates in a positive autocrine ring cytokine, which accelerates release of cytokines from mast cells (Coward *et al.*, 2002).

Although no correlation was found between serum levels of TNF- $\alpha$  with spirometric parameters such as FVC, FEV1, FEV1 / FVC in this study, several scientific resources supported increased expression or levels of TNF- $\alpha$  in respiratory pathways in asthmatic patients (Ying *et al.*, 1991; Bradding *et al.*, 1994). Inhalation of TNF- $\alpha$  leads to hyper response of respiratory pathways and increased neutrophil respiratory pathways in normal subjects (Coward *et al.*, 2002). It is also found out that consumption of TNF- $\alpha$  lead to hyper response of respiratory pathways in asthmatic patients (Thomas *et al.*, 2005; Adner *et al.*, 2002). Although the major mechanisms responsible for this response are not yet fully known, these findings typically support direct effect of TNF- $\alpha$  on smooth muscles of respiratory pathways (Deetz *et al.*, 1997). Many inflammatory effects and features of inflammatory cytokine observed in respiratory diseases and asthma are the same as those of TNF- $\alpha$ . Several studies addressed that TNF- $\alpha$  as an inflammatory cytokine affects numerous processes involved in the pathophysiology of asthma (Hotamisligil *et al.*, 1993; Vgontzas *et al.*, 1997; Zhang *et al.*, 2001). It is reported that an increase in inflammatory cytokine is associated with increased Adhesin and eosinophils synthesis from epithelial cells (Godding *et al.*, 1995). This in turn leads to release of autoxin, which affects eosinophils involved in pulmonary fibroblasts (Sato *et al.*, 2001) and epithelial cells (Lilly *et al.*, 1997; Koyama *et al.*, 1999). It also causes the secretion of IL-6 from eosinophils (Gounni *et al.*, 2000), hyper production of IL-8 by alveolar macrophages (Cromwell *et al.*, 1992) and bronchial epithelial cell damage (Kampf *et al.*, 1999) and activation of endothelial cells (Bjornsdottir *et al.*, 2001) and bronchial stenosis (Martin *et al.*, 2001). Despite relatively sufficient resources for the role of TNF- $\alpha$  in asthma, the findings obtained in this study showed no association between inflammatory cytokine with FVC, FEV1, FEV1 / FVC as respiratory function indices and diagnostic criteria for asthma severity. It is likely that TNF- $\alpha$  affects other inflammatory factors indirectly; as a result, it directly affects respiratory function parameters and severity of the disease. Lack of association between these variables may be attributed to small sample size.

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