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POSITIVE CORRELATION OF CIRCULATING TUMOR NECROSIS FACTOR-ALPHA (TNFα) WITH SPIROMETRIC PULMONARY TESTS IN ADULT ASTHMATIC PATIENTS

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ABSTRACT : Bronchial asthma is a multidisciplinary disease characterized by bronchial inflammation/hyperresponsiveness, in which, more than a few pro-inflammatory mediators and cells take apart. This study includes 96 asthmatic adults divided according to levels of asthma-control into good control, partial control and poorly controlled asthma. Likewise, patients further divided into two groups (treated and untreated groups) depending on whether they were on regular treatment and irregular (or untreated). For each patient; the calculation of anthropometric and spirometric tests including FEV1, PEF, FVC and FEV1/FVC were evaluated. Measurements of TNFα were assessed at the laboratory of Biochemistry in College of Pharmacy, University of Babylon. Results show that the mean patients' age was 34.3±13.5, the lower ages were reported in poorly controlled asthmatics with male/female ratio of 3/2. The mean BMI, mean asthma duration and the total males' number were: 29.6±7.6, 10.5±8.6 and 56 sequentially. Higher TNFα levels observed in poorly controlled asthma (p-0.034), with significant lower spirometric tests of FEV1%, FVC and PEF, which is not the case for FEV1/FVC. There were significant differences in TNFα levels (p-0.001) between those on steroids (systemic or inhaled) therapy and those who were not. In conclusion, the levels of circulating TNFα are higher in poorly controlled asthmatic patients with significant positive correlation to the levels of FEV1 and FEV1/FVC tests.

Key words : Circulating, Tumor Necrosis Factor-Alpha (TNFα), spirometric pulmonary tests, adult asthmatic patients.

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INTRODUCTION

Bronchial asthma (BA) is a diverse disease characterized by bronchial inflammation/ hyperresponsiveness. Universally, BA is one of the most common chronic disorders, affecting around 334 million according to a report from the Global Asthma Network (Al-Aaraji and Baay, 2019), in which, more than a few pro-inflammatory mediators and cells take a part especially eosinophils, mast cells and T lymphocytes. Inflammation of respiratory tracts is supposed to be the chief basis for repeated events of bronchoconstriction in BA (Carr *et al*, 2018; Said *et al*, 2019).

Tumor-necrosis-factor (TNF) is a lymphokine, which occurs in either 'α' or 'β' form (Ewadh and Albayati, 2014). TNFα is a versatile cytokine, can orchestrate the chronic inflammation and histopathological changes of the airway-passages during BA in response to multidisciplinary stimuli like infections and injuries induced by (mast cell, macrophage, neutrophil and eosinophil), besides epithelial cells. TNFα can induce both tissue buildup and stimulation of neutrophils and eosinophils, to be increased in the air-passages in severe BA (Carr *et al*, 2018). Still, insufficient studies have analytically inspected the levels of proinflammatory cytokine levels in BA (Jatakanon *et al*, 1999; Ai-Xia *et al*, 2016). Consequently, it is reasonable to postulate that TNFα blood levels may be closely correlated to the severity of inflammation in the airways of BA. This is the background for conducting this analysis, which aimed to inspect the

serum levels of TNFα and assess its correlation to FEV1 and FEV1/FVC in Iraqi asthmatics. There were no significant gender disparities other than PEF and FEV1/FVC which were higher significantly (p-0.001 and p-0.04 sequentially) in males. There was a significant correlation between TNFα and spirometric tests in terms of both FEV1% and FEV1/FVC (p-0.002) and (p-0.001) consecutively.

MATERIALS AND METHODS

Study participants : This is a cross-sectional study that includes 96 asthmatic adults divided according to levels of asthma-control into three groups: good control asthma (n=22), partial control asthma (n=30) and poor control asthma (n=44). The BA patients were carefully chosen from those attending the respiratory care clinic at Al-Imam Al-Sadiq Teaching Hospital, being free of the chest or systemic infections and their diagnosis was specialists physician. The diagnosis of BA was evaluated by using the Global Initiative for Asthma guidelines (GINA). The anthropometric measurements were taken for the patients and their BMI was calculated as kg/m^2. Likewise, patients further divided into two groups (treated and untreated groups) depending on whether they were on regular treatment and irregular (or untreated) for BA. Treatment included inhaled and systemic steroids, oral and inhaled short-acting β-agonists, antileukotriene drugs, and combined types of inhalers.

Serum TNFα-Immunodetection : The blood samples from all asthmatic patients have been drawn, and the serum was collected after being centrifuged, to be stored at –20°C till the time of biochemical immunoassay of TNFα. The measurements of TNFα were assessed at the laboratory of Biochemistry Department in College of Pharmacy, University of Babylon, by using ELISA kit from "Human TNFα (Tumor Necrosis Factor-Alpha) kit Elabscience® Biotechnology China", with a detection rate of 7.81-500 pg/ml and sensitivity of 4.69 pg/ml.

Pulmonary Function Test (Spirometry) : The % of predicted forced expiratory volume in one second (FEV1%), peak expiratory flow rate (PEF/L), forced vital capacity (FVC/L) and FEV1/L to forced vital capacity ratio (FEV1/FVC) of all patients were recorded using local spirometry available at hospital respiratory unite (Micro Medical® Spiro, USB).

Statistical analysis : Considering the data distribution contrasts among the groups of patients was achieved using SPSS IBM USA Version-25. Figures are shown as means±SD. Likewise, Spearman's-correlation-test was completed to evaluate the statistical correlation. Whereas a *t*-test was done to determine participants' characteristics between both the treated/untreated groups and gender variations. One-way-ANOVA with "post hoc least significant difference test" utilized to compare the means of more than 2 independent factors among the 3 groups. For association among TNFα values and study parameters, data were scrutinized using linear regression analysis. The sorting accuracy of TNFα was analyzed "under the ROC-curves" for their predictive fitness to distinguish the severity of BA.

Ethical consent : This study was approved by the Babylon University, Babylon Health Directorate and Al-Imam Al-Sadiq Teaching Hospital Ethics Committee and all applicants provided their well-versed permission. This study was completed following "Helsinki-Declaration".

RESULTS

The mean ages of the patients were 34.3±13.5 years, the lower ages were reported in those who are poorly controlled BA, with a higher prevalence of males (male/female ratio was 3/2). Meanwhile, there was no significant correlation of age with spirometric values (results not shown). The mean BMI (kg/m^2), mean duration of asthma in the patients per year, and the total number of males were: 29.6±7.6, 10.5±8.6 and 56 sequentially. The main finding in our work was higher TNFá levels observed with the worsening of asthma control significantly (*p*-0.034). Contrariwise, spirometric examination exposed that FEV1%, FVC and PEF were significantly lower in poorly controlled asthma, which is not the case for FEV1/FVC (Table 1). By linear regression analyses, there was no significant correlation of BMI with all study parameters (results not shown).

Concerning the impact of gender on the study variables, it was evident that no significant disparities other than PEF and FEV1/FVC which were higher significantly (*p*-0.001and *p*-0.04 sequentially) in males (Table 2).

To evaluate the differences of the study variables between treated and untreated BA, Table 3 was designed. It showed that there were no significant statistical variations in all study parameters apart from the lower ages of untreated asthmatic patients (*p*-0.028). The second main finding in this work is a significant statistical correlation between TNFα and spirometric tests in terms of both FEV1% and FEV1/FVC (*p*-0.002) and (*p*-0.001) consecutively (Table 4).

Plasma TNFα were further analyzed by the area under the curve statistics for their predictive capability to distinguish poorly controlled asthma analyzed by the receiver operating characteristic curves, that revealed TNFα cutoff value accuracy, specificity, sensitivity,

Table 1 : Characteristics of study variables of study participants according to asthma control levels.

Mean±SD	Number	Duration years	Female sex	Age/years	BMI Kg/m²	TNFá Pg/ml	FEV1% predicted	PEF/L	FVC/L	FEV1/FVC
Total	96	10.5±8.6	40	31.9±15.1	28±6.6	55.1±91.4	77.6±21.2	65.6±22.3	1.12	71.4 ±11.9
Good Control	22	13±10.3	11	36.9±15.8	29.9±5.6	23.6±27.5	92.4±16.2	88.2±18.8	1.33	74.9±7.4
Partial Control	30	10.8±9	11	33.3±13.1	26.3±6.7	51.5±81.7	77.2±16.3	64±16.5	1.12	70.4±8
Poor Control	44	7.3±1.1	18	28±15.1	28.1±6.9	73.5±112.8	71.18±22.7	55.8±18.9	1.01	70.3±15
Significance		0.3	0.01	0.018	0.47	0.036	0.001	0.001	0.001	0.17

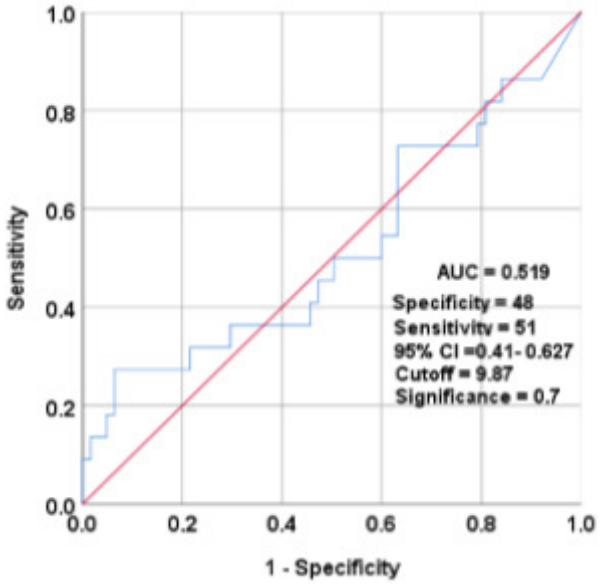

Fig. 1 : ROC analysis of TNF-α and levels of asthma severity in asthmatic patients.

significance and 95% CI as follow: 9.87 pg/ml, 51%, 48, 51, 0.7 and 0.41- 0.62 sequentially. There were significant differences in TNFα levels (*p*-0.001) between those on steroids (systemic or inhaled) therapy and those who were not (results not shown).

DISCUSSION

Cytokines are dynamically orchestrating the inflammatory cells' infiltrate of the bronchial tree in BA and their physiological pathways inside the respiratory tissues. Such inflammatory invasion comprises primarily eosinophils and lymphocytes. TNFα is a strong cytokine, has an extensive pro-inflammatory activity that may augment allergic reactions in the body. TNFα is well-known to upsurge bronchial hyperresponsiveness (BH) when administered as an inhaled aerosol (Waserman *et al*, 2000). Several (old and recent) underlying revisions, arguments in favor of the correlation between TNFα and inflammatory pulmonary disorders (David *et al*, 1992; Singh *et al*, 2018; Gonzalez-Jaramillo *et al*, 2019). On the same vein; this survey is an attempt to inspect the correlation of serum levels of TNFα as an inflammatory lymphokine with pulmonary function tests.

There is a controversial correlation of BA with the increasing age of asthmatic patients (Shinya *et al*, 2005; Denlinger *et al*, 2017; Al-Aaraji and Baay, 2019). Although, the mean age of three asthma groups in our study was not critically diverged, it is uncertain that this element adversely disturbs the interpretation of this work because there were no differences in whole study variables according to age. In support of our findings, two recent analyses reported the same outcomes (Denlinger *et al*, 2017; Al-Aaraji and Baay, 2019).

Table 2 : Differences of study variables according to the gender of both study groups.

Gender	Age/years	BMI (kg/m^2)	Duration	TNFá	FEV1	PEF	FVC	FEV1/FVC
Female N=40 (Mean±SD)	34.4±14.3	28.4±5.2	10.5±7.2	94.2±200.2	84.9±21.1	71.1±23.5	1.14	75.3±11.2
Male N=56 (Mean±SD)	33.9±12.7	30.6±9.1	10.4±10.9	79.3±117.2	88.7±17.6	82.9±22.9	1.14	78.9±11.2
Significance	0.7	0.042	0.9	0.54	0.19	0.001	0.9	0.04

Table 3 : Differences of study variables between treated and untreated asthmatic patients.

	Number	Age	BMI	TNFá	FEV1	PEF	FVC	FEV1/FVC
Treated	60	34.3±14.6	27.4±5.8	54.9±104.4	81.2±21.9	68.3±23.3	1.16±0.3	73.5±11.5
Untreated	36	27.4±14.8	29±7.6	55.7±65.6	72.4±18.5	61.5±19.4	1.06±0.3	67.6±11.9
p		0.028	0.24	0.96	0.48	0.14	0.17	0.95

Table 4 : Correlation of TNFá to the study variables by logistic regression analysis in study populations.

	Duration	Age	BMI	FEV1	PEF	FVC	FEV1/FVC
r	-0.09	0.07	-0.053	0.22	0.019	0.002	0.29
p	0.36	0.34	0.47	0.002	0.89	0.89	0.001

Two current studies in Babylon province (Al-Aaraji and Baay, 2019) further support the notion of our study, displayed no significant correlations of BMI with all study variables among the three asthma groups except gender. In all three studies, the female asthmatics were to a certain extent heavier. In contrast, three recent studies revealed contradictory results; first: revealed a strong correlation between asthma control test and BMI among obese BA (FM, 2017), second: obese females had higher rates of BA than obese males then; these rates were exaggerated by smoking habits (Greenblatt *et al*, 2017). The third displayed a significantly higher mean BMI in asthmatic patients compared to the healthy group (Singh *et al*, 2018). Communally, these revisions highpoint the significance of BMI during assessing BA and the necessity for supplementary researches in these fields to sightsee inconsistencies among analyses.

One more thing should be pointed out in this work, which is the significant sex variation of spirometric measures. The data yielded provides convincing evidence that males have higher values of PEF and FEV1/FVC mutually. Overwhelming pieces of evidence are corroborating this notion in the current era (LoMauro and Aliverti, 2018; Naeem and Silveyra, 2019; Amjed *et al*, 2021).

The basic mechanisms beyond the gender difference in the incidence of BA are multifactorial, but still not known precisely. Both hormonal and/or pulmonary-capacities biomodifications among two sexes can be anticipated as contributing masterclasses (Brown *et al*, 1986; LoMauro and Aliverti, 2018). Oestrogen receptors are extensively spread in immune-cells, which associate with oestrogen and contribute to allergic reactions thereby influencing immune response, cytokines, and proinflammatory factors (Fan *et al*, 2019). The impact of obesity as a variable risk issue of BA may have further influences on females' spirometry (Greenblatt *et al*, 2017). Moreover, lower plasma levels of vitamin D3 may be associated with the proinflammatory cytokines IL-6 and TNFα, besides altered lung functions (Naeem and Silveyra, 2019; Wang *et al*, 2018). Last of all one can add other influential factors like altered aspirin sensitivity in females (Salman, 2012), high exposure for domestic cooking gas in women (Jarvis *et al*, 1996; Vincent *et al*, 2017).

The correlation of TNFα values with the reading of spirometric tests' lies at the heart of the discussion. This study hypothesized that blood TNFα as an inflammatory marker in BA could be used to differentiate good from poorly controlled BA. On logical grounds, there is no compelling reason to argue that TNFα values are significantly increased with the worsening of the asthma levels. TNFα revealed a highly significant, positive correlation with FEV1 and FEV1/FVC ratio (*p*-0.002 and *p*-0.001, respectively). There are rapidly growing pieces of literature on BA, which indicates the contributory role of TNFα in BH (Ewadh and Albayati, 2014; Majak, 2016; Suhana Ahmad, 2018,). Conversely, plasma TNFα levels did not significantly differ between asthmatics and control groups in other surveys (AL-Saadi, 2019), whereas TNFα negatively related to FEV1 and FEV1/FVC in other analyses held at 2016 (Ai-Xia *et al*, 2016).

The rationale for inspecting the correlation of TNFα with asthma severity is robust. First of all, several

pathophysiological pathways that control BA refractoriness, including high neutrophilic inflammation, "exaggerated remodeling", steroid resistance, besides systemic inflammation can be clarified by amplified air passages' synthesis of TNFα (Heaney and Robinson, 2005). Next, the reasons associated with the progression of asthma severity including cigarette-smoke, endotoxins exposure, obesity and long-lasting infections are associated with activation of the innate-immunity and hence likely they increase TNFα synthesis (Afrah *et al*, 2017; Jaakkola *et al*, 2019; Shamsollahi *et al*, 2019; Hamdan *et al*, 2019). Thirdly, usage of "synthetic inhaled TNFα" leads to BH in patients with BA as reported in few works (Thomas and Heywood, 2002).

Most of the data about the precise role of TNFα in BA is thus far to be resolute. TNFα as a pro-inflammatory is lymphokines that assist WBCs (eosinophils) recruitment through enhancement of the adhesions molecule on vascular endothelium and training of lymphokine synthesis, BH and airways-remodeling (Suhana, 2018). TNFα has a role in epithelial-barrier dysfunction by adhesions molecule (p120 and E-cadherin) and rising permeability of vascular endothelium to multiallergens (Suhana *et al*, 2018). In bronchial smooth muscle cells, TNFα-dependent-hyperplasia and vasospasm are imperative. In refractory BA, it is expected to find smooth-muscle-hyperplasia plus the invasion of inflammatory cells and hyperexpression of a group of cytokines like IL-4, 13, 1β and TNFα (Srirupa, 2006). The later may modify the expression of membrane receptors like CD40 and OX40 on smooth muscle cells (Burgess *et al*, 2005). In allergic BA TNFα "mediated through 2-receptors: TNFR-1 and TNFR-2" is mostly intricated in the augmentation of "mast cell-associated" histamine secretion. The role of Th1, 2, 9, 17, 22, as well as other cell types are also intricated in allergic BA (Suhana *et al*, 2018).

Several previous researches have revealed an association of certain growth factors with BA. Platelets-derived growth factor is a glycoprotein (Fouad *et al*, 2020; Hayder *et al*, 2020) affects bronchioles during BA inducing divion of smooth muscle cells, wit increased collagen production by pulmonary-fibroblasts (Kardas, 2020). As well, other potent pro-fibrogenic "transforming growth factor-β (TFG-β) (Al-Hindy and Shaker, 2020) overexpressed in the asthmatic bronchioles and is a foremost candidate for the provocation and persistence of airway-remodeling (Boxall and Daviess, 2006). Clinical reports have shown a double role for TGF-β, either as a pro- or anti-inflammatory cytokine, sharing in the initiation and resultion of immune responses of the airways (Duvernelle and Frossard, 2003).

There is an unmet necessity to develop novel analytical biomarkers for clinical evaluation of BA for both prognostic and therapeutic purposes. Of note, even if BA is regarded as eosinophilic-pathology, on another extreme of the illness spectrum; there is a different inflammatory profile involving neutrophils. This could enlighten the "unsuitable response" of some BA to classical asthma therapies, since neutrophilic-inflammation (unlike eosinophilic-inflammation) may resist steroid therapy. Henceforward, the assortment of subjects with noneosinophilic BA may have generated diverse outcomes, as patients with eosinophilic BA had lower TNFα measures from sputum cells or blood compared to patients with noneosinophilic BA (Quaedvlieg *et al*, 2005; Holgate *et al*, 2011).

CONCLUSION

The levels of circulating TNFα are higher in poorly controlled asthmatic patients with a significant positive correlation to the levels of FEV1 and FEV1/FVC tests.

Limitation

Such a decision perhaps overvalued due to rather a low number of observations. Similarly, multivariate logistic regression analysis, which allows concluding on the independence of associations, usually demands a significantly higher sample size. Another likelihood is that the people studied may encompass a higher percentage of patients whose intractable symptoms were triggered by non-asthma related issues. Other important supplementary measurements are required include measurement of neutrophils count, measurement of CRP, and measurement eosinophils (atopic or not).

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