The Relationship between Expressions of Toll-Like Receptor 4 and BODE Index Score in Patients with Chronic Obstructive Pulmonary Disease

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INTRODUCTION
Chronic obstructive pulmonary disease (COPD) is a group of common medical disorders. Morbidity and mortality rate of this disease are increasing due to lack of both effective treatment and full understanding of pathogenesis and mechanism of this disease. Thus, current study aims to investigate the relationship between the expression of toll-like receptor 4 (TLR4) and BODE index in patient with COPD as a part of potential mechanism in the developing of COPD. This study is a case control study conducted in both AlSadar and Almerjan hospitals. A total of 187 individuals (87 patients with COPD and 100 people without COPD) were selected after application of inclusion and exclusion criteria. Using RT-PCR, the expression of TLR4 was investigated. FEV1/FVC ratio was examined using spirometry. There was a significant decrease in the expression of TLR4 among COPD patients when compared with healthy individuals (P < 0.001). COPD patients showed a significant increase in BODE index when compared with control people (P< 0.001). The decrease in the level of TLR4 expression in association with increased BODE index might consider as a suitable indicator for stability and chronicity of the disease. The pulmonary function test is still convenient method for screening and diagnosis of COPD.

Keywords: Toll-like receptor 4, BODE index score, chronic obstruction pulmonary disease

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MATERIALS AND METHODS
Study design, settings and duration
This study was a case control study conducted in respiratory consultancy clinic of Al-Sadar Teaching Hospital at Al-Najaf governorate / Iraq and Merjan Teaching Hospital at Babylon governorate / Iraq during the period January-December 2018, sample size included87 patients with COPD and 100 healthy people. After application of demographic data and inclusion and exclusion criteria, BMI was measured, and blood samples were obtained to assess the expression of TLR4.FEV1/FVC ratio was examined using spirometry.
### Statistical analysis

All data are either mean ± SD or frequencies (as percentages) using SPSS, version 24. Multiple contingency tables were conducted, and appropriate statistical tests performed. Chi-square test was used for categorical variables (Fishers exact test was used when total expected variables were less than 20%). Independent sample t-test was used to compare between two means. Statistical significance was considered for P < 0.05.

### RESULTS

#### Demographic distribution of Age, gender and body mass index of study participants

There was no significant difference in mean age between COPD patients and healthy people. Moreover, no significant differences were observed between COPD patients and control people regarding their gender. Furthermore, there was no significant difference in mean BMI between COPD patients and control individuals, see table (1).

#### Spirometry findings of study groups

There was a significant decrease of FEV1/FVC ratio among COPD patients when compared with healthy people (p<0.001; OR=33.4) (Table 2).

#### Expression of toll-like receptor 4

The TLR4 expression was significantly decreased in COPD patients when compared to healthy individuals (p=0.001; OR=1.6) (Table 3).

#### BODE index score

The BODE index score of COPD patients was classified into four quartiles; quartile 1 (0-2) was represented by 48 (55.2%) COPD patients, quartile 2 (3-4) was represented by 21 (24.1%) COPD patients, quartile 3 (5-6) was represented by 11 (12.6%) COPD patients and quartile 4 (7-10) was represented by 7 (8.1%) COPD patients, while all controls were belonging quartile 1 BODE index score. There was a significant association between higher BODE index score and COPD patients (p < 0.001), table (4).

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**Table (1):** Demographic distribution of age, gender and body mass index of study groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>COPD Mean ± SD*</th>
<th>Control Mean ± SD*</th>
<th>P value*</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>46.5±9.48</td>
<td>45.1±11.4</td>
<td>0.38</td>
<td>1.6</td>
<td>(1.74-4.5)</td>
</tr>
<tr>
<td>BMI</td>
<td>28.1±4.5</td>
<td>26.7±5.1</td>
<td>0.07</td>
<td>1.3</td>
<td>(0.1-2.7)</td>
</tr>
<tr>
<td>Gender</td>
<td>Male 75(96.2%)**</td>
<td>95(95%)**</td>
<td>0.7</td>
<td>1.3</td>
<td>(0.3-5.6)</td>
</tr>
<tr>
<td></td>
<td>Female 3 (3.8%)**</td>
<td>5 (5%)**</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Independent sample t-test, OR=Odds Ratio, CI=Confidence Interval.  
** Fishers exact test, OR=Odds Ratio, CI=Confidence Interval.

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**Table (2):** Distribution of FEV1/FVC according to COPD patients and controls

<table>
<thead>
<tr>
<th>Variable</th>
<th>COPD Mean ± SD</th>
<th>Control Mean ± SD</th>
<th>P value*</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV1/FVC</td>
<td>48.7±3.6</td>
<td>82.4±3.04</td>
<td>&lt;0.001</td>
<td>-33.4</td>
<td>(6.4 - -)</td>
</tr>
</tbody>
</table>

*Independent sample t-test, FEV1=First Second of Forced Expiration, FVC=Forced Vital Capacity, OR=Odds Ratio, CI=Confidence Interval.

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**Table (3):** Distribution of TLR4 according to COPD patients and controls

<table>
<thead>
<tr>
<th>Variable</th>
<th>COPD Mean ± SD</th>
<th>Controls Mean ± SD</th>
<th>P value*</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLR4</td>
<td>10.3±3.8</td>
<td>11.9±2.8</td>
<td>0.001</td>
<td>-1.6</td>
<td>(-2.6 - -0.6)</td>
</tr>
</tbody>
</table>

*Independent sample t-test, OR=Odds Ratio, CI=Confidence Interval.

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**Table (4):** Distribution of BODE index score according to COPD patients and controls

<table>
<thead>
<tr>
<th>Variable</th>
<th>COPD No.</th>
<th>COPD %</th>
<th>Control No.</th>
<th>Control %</th>
<th>P value</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>BODE index score</td>
<td>48</td>
<td>55.2</td>
<td>100</td>
<td>100</td>
<td>&lt;0.001</td>
<td>1.3</td>
<td>(0.3-5.6)</td>
</tr>
<tr>
<td>Quartile 1 (0-2)</td>
<td>21</td>
<td>24.1</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quartile 2 (3-4)</td>
<td>11</td>
<td>12.6</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quartile 3 (5-6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
DISCUSSION
The chronic obstructive pulmonary disease is anticipated to be in the top list of death causing diseases all over the world at 2030 [17]. COPD is associated with a gradual limitation of airflow and stimulated chronic airways inflammation [18]. The inflammatory markers and classifications of severity for patients with COPD are all aimed to help physicians to predict patients at risk for early treatment in attempt to decrease the morbidity and mortality rates [19]. The prevalence of smoking in Iraq has increased in post-conflicts after 2003 and this higher rate had been attributed to political reasons, capacity building and technical reasons and law enforcement reasons [20]. Smoking in young people is strongly linked to early development of COPD and earlier co-morbidities of COPD and death [21]. Current study showed no significant difference in age between COPD patients and healthy people. This finding is essential in excluding the confounding effect of age on results of this study. It was found that the age is an important risk factor for development and severity of COPD [22]. In Iraq, because of traditions and religious restrictions, smoking of women is considered as social stigmata which fortunately reduced the behaviour of smoking among Iraqi women [23]. In the UK, a current study carried out by Peters et al (2014) reported that differences between both men and women regarding smoking behaviour had been diminished in last decades as there was a rise in smoking prevalence among women. Many researchers suggested that the risk of long duration smoking is higher among women in comparison to men. Some studies reported that the lung cancer probability was increased among women with equal smoking/packs per year as men leading to fact that smoking is riskier in women than men [24-25]. The inverse relationship between BMI and COPD is also found in other chronic diseases like type 2 diabetes mellitus [26] cerebrovascular accidents [27] and chronic renal disease [28]. Till now, this inverse relationship between mortality of COPD and BMI is controversial and dose-response relationship [29]. For normal non-smoker population, the decline in FEV1 is approximately ranging between 30-50 ml per year [30]. When the rate of decline is higher than this rate, it is regarded as an abnormal rapid decline. High suspicion was around the selection of better threshold level of forced expiratory ratio and using it for diagnosis of COPD as these forced expiratory ratios are declined with age in normal population [31]. This may lead to false positive diagnosis of normal elderly peoples as COPD [32]. Additionally, the classification of severity of COPD based on FEV1 might also be misclassifying the COPD among elderly population as misclassifying elderly patients in GOLD stage I as GOLD stage II [33], although these limitations of FEV1, all international guidelines apply forced expiratory ratio for COPD [2]. Present study showed low level of TLR4 among COPD patients when compared to healthy individuals. This result might be due to activity or chronicity of the disease. This is relatively in line with data conducted in a study in Netherlands which reported TLR4 level of 10.8 ng/µL among COPD patients [34], this finding is consistent with results of a study in South Korea which enrolled 53 smoker patients and found that FEV1/FCC ratio was significantly increased with the increase of TLR4 level, while emphysema score is decreased with the increase of TLR4 level [35]. Data of this study were inconsistent with results of a study conducted in India which found that gene expression of TLR2 and TLR4 were increased in lip polysaccharide-stimulated peripheral blood Neutrophils of COPD. These differences in studies are explained by a study conducted in Sweden which found that TLR2 level is decreased in sputum Neutrophils of COPD while there was no significant difference between smokers with COPD and smokers without COPD regarding TLR4 expression. They reported that TLRs are the first line of defence against invading microorganisms, but they are differently regulated in smokers with COPD compared with smokers without COPD. Nowadays, many studies found a strong association between innate immunity and COPD. Innate immunity included phagocytes of inflammatory cells that associate between pathogens and self-cells by using signals from TLRs. These receptors are essential in COPD, since they have an important defensive role against viral and bacterial infections, and these infections of airways will exacerbate COPD disease [37]. TLR4 plays a role in lung homeostasis through participating in defensive mechanism of endothelial cells against oxidants [38]. The BODE index score of COPD patients in current study was classified into four quartiles; quartile 1 (55.2%), quartile 2 (24.1%), quartile 3 (12.6%) and quartile 4 (8.1%). These findings are in line with the results of a study which revealed that BODE index scoring of older COPD patients is precise in prediction of risks and mortality and they showed that about 50% of COPD patients were in quartile 1 [39]. Current study showed a highly significant association between higher BODE index score and COPD patients. This is in agreement to results of studies which documented that BODE index score was predominantly increased in COPD patients with high risk of mortality [40-41]. Another study found that BODE index was strongly related to health-related quality of life in patients with stable COPD and the BODE was better than Gold staging of COPD in prediction of COPD severity [42]. Thus, many researchers strongly advise the multiple applications of BODE index for evaluating health and predicting morbidity and mortality risk of COPD patients.

CONCLUSION
TLR4 expression in patients with COPD is declined, implicating an important role in diagnosis of chronicity of obstructive pulmonary disease. Additionally, the BODE index is the best readout for severity assessment and mortality of COPD. Moreover, the decreased level of TLR4 in association with increased of BODE index might be considered as a good indicator for chronicity of disease. Finally, the pulmonary function test measurement of forced expiratory ratio is still easiest method for screening and diagnosis of COPD.

| Quartile 4 (7-11) | 7 | 8.1 | 0 | - |

* Fishers exact test, OR=Odds Ratio, CI=Confidence Interval.
REFERENCES


