Effect of hypothyroidism on ova albumin-sensitized guinea pigs: An observational a cross-sectional study

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ABSTRACT

Background: The relationship between asthma and thyroid disease provides indirect evidence for a role of thyroid hormones in maintaining airway function. Thyroid disease and asthma, both being common conditions, occasionally occur together. Objectives: to investigate the effect of hypothyroidism on the level of tumor necrosis factor and total Leucocytic count in ova albumin-sensitized guinea pigs. Material and methods: twenty-four male guinea pigs were assigned randomly into two equal groups. Group (1) represents euthyroid while group (2) represents induced hypothyroid group that induced by methimazole in drinking water. The level of tumor necrosis factor and total Leucocytic count analysis of bronchoalveolar lavage fluid (BALF) were measured for both groups. Results: Statistical analysis revealed a significant increase of the level of tumor necrosis factor in favour to group (2) in compared to group (1) while there was no significant difference in total Leucocytic count between both groups. Conclusion: Hypothyroid groups had higher level of tumor necrosis factor than euthyroid group which reflects worsening of asthma in hypothyroidism.

Key words: Bronchial asthma, hypothyroidism, ovalbumin, sensitized guinea pigs.

Introduction

Asthma is a chronic inflammatory condition defined primarily by clinical characteristics; it is often considered to be a syndrome rather than a disease, as patients exhibit a range of different phenotypes including differences in the type of inflammation or response to therapy (Agache et al., 2012). The relationship between asthma and hypothyroidism provides indirect evidence for a role of thyroid hormones in maintaining airway function. Thyroid disease and asthma, both being common conditions, occasionally occur together. Development of hypothyroidism (HOT) may ameliorate coexistent asthma (Manzolli and Vianna, 1999) conversely, treatment of the HOT state may result in worsening airway obstruction (Wieshammer et al., 1990). However, on the other hand others declared that giving minute amount of thyroxine in patients with bronchial asthma improved the airways response to the bronchodilator medications (Abdel Khalek et al., 1991) and that decreased T3 level contribute to mucus hypersecretion (Wang et al., 2012). To the author’s knowledge, there has been no previous study which evaluated the effect of hypothyroidism on ova albumin-sensitized guinea pigs. Therefore, the main purpose of this study was to investigate the effect of hypothyroidism on the level of tumor necrosis factor and total Leukocyte count in ova albumin-sensitized guinea pigs.

Materials and methods:

Design:

This was a cross-sectional study aiming to assess the impact of hypothyroidism on the level of tumor necrosis factor and total Leukocyte count in guinea pigs.

Animals:

Twenty-four adult male guinea pigs (weight 250 – 350 g), were housed under a 12h light/dark cycle. The animals had free access to food and water throughout the experiments. Sensitized animals

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were randomly grouped into 2 main groups each group 12 guinea pigs; (Group (I): euthyroid group and Group (II): hypothyroid group). Hypothyroidism was induced by methimazole in drinking water (50 mg/kg b.w. daily for 7 weeks) (Lewinson et al., 1994). All experiments followed for guidelines for the Care and Use of Laboratory Animals 8th Edition (2011) that is adopted by the Institutional Animal Care and Use Committee (IACUC) of Cairo University.

**Instrumentation and Tools:**

**I-Drugs and Chemicals:**

- Carbimazole tablets 5 mg (chemical industries development CID).
- Ovalbumin powder (OVA) (Sigma aldrich medical company, Italy) was dissolved in distilled water forming 1%, 2% OVA for sensitization and 2ry challenge of guinea pigs respectively.

**II-Kits**

Guinea pig –specific TNF alpha kit (Sunred Biological Technology Co., Ltd, China).

**Experimental Procedure:**

**Animal Sensitization:**

Animals were sensitized by means of ovalbumin according to the method of (McCaig, 1987) in which animals were injected with 10 mg IP (1 ml) and 10 mg SC (1 ml) on day 1 and 10 mg IP on day 8 to be challenged on day 14.

**Bronchoalveolar lavage:**

At the end of the experiment, bronchoalveolar lavage fluid (BALF) was collected from each animal through a blunt stainless steel cannula with a female Luer hub (14G) was inserted into the trachea, and then warm saline solution (1mL/ 100 gram) was introduced into the lungs via a 10-ml syringe and then recovered 5 min later. The recovered lavaged fluid was centrifuged and the supernatant was collected and stored at − 80 °C (Smith and Broadley, 2010).

**Outcome measures:**

Broncho-alveolar lavaged fluid was employed to test:

1-The level of tumor necrosis factor (TNF α ) in the supernatant of the recovered bronchoalveolar lavaged fluid were measured using enzyme-linked immunosorbent assay (ELISA) kits (Mahajan and Mehta, 2011).

2- Total leucocytic count:

The cell pellets were re-suspended in one milliliter of normal saline. By taking 50 µL from the cellular suspension, the total number of cells was counted by a hematocytometer. (Tripathi et al., 2010).

**Principles of biochemical assay: ELISA:**

The Kits used to measure BAL fluid cytokines are a solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich (indirect) principle. The test specimen (BAL fluid) is added to the tested antigen (TNFα) monoclonal antibodies immobilized on polystyrene microtiter wells (solid phase) and incubated with the Zero Buffer. If the antigen is present in the specimen, it will combine with the antibodies on the well. The well is then washed to remove any residual test specimen, and goat anti-antigen in the antibody-enzyme (horseradish peroxidase) conjugate reagent is added. The conjugate
reagent will bind immunologically to the antigen on the well, resulting in the antigen molecules being sandwiched between the solid phase and the enzyme-linked antibodies. After incubation at room temperature, the solid phase is washed with water to remove unbound labeled antibody. A chromogen solution of 3,3', 5, 5'-Tetramethylbenzidine (TMB) is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped and the resulting yellow color is measured spectrophotometrically at 450 nm. The concentration of antigen is directly proportional to the color intensity of the test sample (Engvall and Perlmann, 1971; Uotila et al., 1981).

Statistical Analysis

Statistical analysis was conducted using SPSS for windows, version 23 (SPSS, Inc., Chicago, IL). The current study involved one independent variable (tested group) that had two levels (group 1 represents euthyroid group and group 2 represents induced hypothyroid group). In addition, this study involved two tested dependent variable (The level of tumor necrosis factor and total Leucocytic count analysis). There were no outliers in the The level of tumor necrosis factor and total Leucocytic count analysis, as assessed by inspection of a boxplot. The level of tumor necrosis factor (TNF $\alpha$) and total Leucocytic count analysis for each level of group were normally distributed, as assessed by Shapiro-Wilk's test ($p > .05$), and there was homogeneity of variances, as assessed by Levene's test for equality of variances ($p > .05$). So, "Unpaired t test" was conducted to compare The level of tumor necrosis factor and total Leucocytic count analysis between both groups with the alpha level 0.05.

Results

The mean ± SD values of TNF $\alpha$ and Total leucocytic count in the "group 1" and "group 2" are presented in table (1) for both groups. "Unpaired t test" revealed that the mean values of the " TNF $\alpha$ " level between both groups showed there was significant differences ($p<0.05$) and this significant increase in favor of group 1. However, the mean values of the " Total leucocytic count " level between both groups showed there was no significant differences ($p>0.05$).

Table 1: Mean ±SD, t and P values of TNF $\alpha$ and Total leucocytic count at both groups.

<table>
<thead>
<tr>
<th></th>
<th>Group 1 Means ± SD</th>
<th>Group 2 Means ± SD</th>
<th>Mean difference</th>
<th>t-value</th>
<th>P- value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF $\alpha$</td>
<td>11.72±1.44</td>
<td>16.51±0.83</td>
<td>-4.79</td>
<td>-7.049</td>
<td>0.0001*</td>
</tr>
<tr>
<td>Total leucocytic count</td>
<td>18105.33±1234.81</td>
<td>19770.33±1461.53</td>
<td>-1665</td>
<td>-2.132</td>
<td>0.06</td>
</tr>
</tbody>
</table>

*Significant level is set at alpha level <0.05.

Discussion

Thyroid disease and asthma, both being common conditions, occasionally occur simultaneously. The relationship between asthma and thyroid disease provides indirect evidence for a role of thyroid hormones in maintaining airway function. (Naeimi et al., 2012). The aim of the present study to investigate the effect of hypothyroidism on the level of tumor necrosis factor and total Leucocytic count in ova albumin-sensitized guinea pigs. In the present work, ovalbumin (OA) model of asthma was used. Guinea pigs were sensitized by means of ovalbumin according to the method of (McCaig, 1987) in which animals was animals were injected with 10 mg IP (1 ml) and 10 mg SC (1 ml) on day 1 and 10 mg IP on day 8 to be challenged on day 14.

Sensitized non-treated guinea pigs showed significant increase in: in BAL fluid total leukocyte count and TNF alpha. The result of the current study revealed that there was significant difference between both groups in TNF$\alpha$ in favor to group 2 (induced hyperthyroid group) in compared to group 1 (euthyroid group).

Ovalbumin sensitized guinea pig model by Xue et al. (2014), the guinea pigs were sensitized with intraperitoneal (I.P.) injection of OVA on day 0 and challenged with aerosolized OVA for six consecutive days 14 days later. The animals were sacrificed 24 hours after the last challenge. They found
significant elevation of inflammatory cytokines (IgE, TNF-α and IL4), eosinophilic percentage in bronchoalveolar lavage fluid and marked infiltration of eosinophils among bronchioles and alveoli. This agree with the result of present study.

Similar to our results, a correlation between hypothyroidism and low-grade inflammation was indicated previously (Abbas and Sakr, 2016). Levothyroxine (L-T4) treatment of hypothyroid rats markedly decreased the elevated serum levels of TNF-α and IL-6. Marfella et al., 2011 also observed significantly lower plasma TNF-α and IL-6 levels in patients with subclinical hypothyroidism treated with L-T4 compared to the untreated individuals. However, only a few studies observed those changes over a prolonged period. The levels of IL-6 and TNF-α increased with longer duration of the I131 treatment. Zhou et al., 2018 showed that at the end of month 2 and 4, when the cytokine pro-inflammatory mediators was similar to the natural background value, the levels of TNF-α and IL-6 were still continually increasing, indicating that the elevated levels of the pro-inflammatory cytokines are probably associated with hypothyroidism. In addition, increased levels of TNF-α and IL-6 have been suggested as a risk factor for adverse cardiovascular events (Abbas and Sakr, 2016).

Conclusion

Hypothyroidism increase tumor necrosis factor alpha level and total leukocytic count in bronchoalveolar lavage in sensitized guinea pigs. The study concluded that in resistant cases of asthma; hypothyroidism should be excluded as it could worsen asthma.

References

