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T Cell Immunity Profile in Pediatric Obesity and Asthma

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***Abstract*—**The mechanisms underlying the association between obesity and asthma may be related to a decreased immunological tolerance induced by a defective function of regulatory T cells (Tregs). The aim of this study is to establish the potential link between these diseases and CD4+, CD25+ FoxP3+ Tregs as well as T helper cells (Ths) in children. This is a prospective case control study. Obese (n:40), asthmatic (n:40), asthmatic obese (n:40) and healthy children (n:40), who don't have any acute or chronic diseases, were included in this study. Obese children were evaluated according to WHO criteria. Asthmatic patients were chosen based on GINA criteria. Parents were asked to fill up the questionnaire. Informed consent forms were taken. Blood samples were marked with CD4+, CD25+ and FoxP3+ in order to determine Tregs and Ths by flow cytometric method. Statistical analyses were performed. $p \leq 0.05$ was chosen as meaningful threshold. Tregs exhibiting anti-inflammatory nature were significantly lower in obese (0,16%; $p \leq 0,001$), asthmatic (0,25%; $p \leq 0,01$) and asthmatic obese (0,29%; $p \leq 0,05$) groups than the control group (0,38%). Ths were counted higher in asthma group than the control ($p \leq 0,01$) and obese ($p \leq 0,001$) groups. T cell immunity plays important roles in obesity and asthma pathogeneses. Decreased numbers of Tregs found in obese, asthmatic and asthmatic obese children may help to elucidate some questions in pathophysiology of these diseases. For HOMA-IR levels, any significant difference was not noted between control and obese groups, but statistically higher values were found for obese asthmatics. The values obtained in all groups were found to be below the critical cut off points. This finding has made the statistically significant difference observed between Tregs of obese, asthmatic, obese asthmatic and control groups much more valuable. These findings will be useful in diagnosis and treatment of these disorders and future studies are needed. The production and propagation of Tregs may be promising in alternative asthma and obesity treatments.

***Keywords*—**Asthma, flow cytometry, pediatric obesity, T cells.

I. Introduction

IN recent decades, asthma and obesity prevalence have increased and reached epidemic proportions. Obesity is a common comorbidity to asthma [1], [2]. In a study performed among the children with asthma, multivariable analysis identified obesity to be associated with uncontrolled disease [3]. Overweight/obese children with early-onset asthma have been reported to display poorer asthma control and a distinct pattern of symptoms [4]. Overweight and obesity both have been shown to be strongly related to wheeze. There was also a clear association of overweight and obesity with an objective marker of airways obstruction (FEV1/FVC) [5]

Although the association between obesity and asthma is very clear, many questions still exist in regard to this relationship. Epidemiologic studies have supported the link between these two diseases. Genetic factors are also involved in the development of both obesity and asthma. Biological interactions including hormonal influences as well as chronic systemic inflammation appeared to be involved in this connection [1]. Among cells of the immune system, T cells play a major role in the inflammatory response [6]. Experimental studies have shown that T regulatory cell (Treg) levels alter tissue inflammation and systemic metabolic parameters, pointing to Tregs as negative regulators of pro-inflammatory responses in obesity [7]-[10]. Activation of immune cells is closely associated with insulin sensitivity. Studies using flow cytometry subsequently identified the relative importance of immune cells, including T cells during the development of chronic inflammation. Contributions of inflammation to the development of insulin resistance and subsequent metabolic abnormalities are being investigated [11].

The aim of this study is to evaluate the status of Tregs and T helper cells (Ths) in obese, asthmatic and obese asthmatic children in comparison with those of healthy children and their possible relations with the status of the insulin sensitivity index; Homeostasis Model Assessment of Insulin Resistance (HOMA-IR), in children with obesity as well as in obese children complicated with asthma.

II. Materials and Methods

A. Patients

A total of 160 children; 40 healthy, 40 obese, 40 asthmatic children and 40 children with obesity and asthma were included into the scope of this study. Their ages, weights, heights, waist and hip circumferences were measured and body mass index (BMI) values were calculated. WHO BMI values for 0-5 year-aged-children [12] and BMI values designed for 5-19 year-aged-children and adolescents [13] were used as the values for determining the obesity criteria. The values obtained from the clinical examination were compared with the international reference data including

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percentile curves. Asthmatic children were evaluated performing clinical examination, pulmonary function tests and questionnaires. They were chosen based on GINA criteria. The children from three patient as well as control groups did not have any additional diseases and related treatments. Blood samples from the patients and controls were obtained under the protocols approved by the Namik Kemal University, Faculty of Medicine, Ethical Committee for Clinical Investigations. A written, signed informed consent was obtained from the parents of the participating children. Procedures were carried out in accordance with Declaration of Helsinki.

B. Flow Cytometry Analysis

Blood samples drawn from the children participating in the study were collected into EDTA-containing tubes. Peripheral blood lymphocytes were isolated through Ficoll density gradient centrifugation. Tregs were defined as cells, which were CD4+, CD25+ and FoxP3+. Cells were first incubated with fluorochrome-conjugated monoclonal antibodies against surface antigens, anti-CD4-FITC and anti-CD25-APC followed by the steps during which cells were fixed, permeabilized and intracellularly stained with anti-FoxP3-PE. Multicolor immune fluoresence analysis was performed using BD FACSCalibur flow cytometer and CellQuest software. The evaluation of the Treg subpopulations by flow cytometry was determined as the frequency of CD25+FoxP3+ expressing cells within the CD4+ compartment.

C. Laboratory Investigations

Blood samples were obtained from the children for glucose, insulin and routine analyses. Plasma was separated by centrifugation.

Fasting blood glucose levels were measured by spectrophotometric hexokinase assay; fasting insulin levels were determined by ECLIA (electro-chemi-luminescence immunoassay).

HOMA-IR [14] was calculated using fasting plasma glucose and insulin values [HOMA-IR= fasting glucose (mg/dL)* fasting insulin (μIU/ml)/ 22,5*0,0555)] to estimate IR. The cut-offs 3.16 [15] and 2.5 [16], [17] were considered for HOMA-IR.

D. Statistical Analyses

Statistical analyses were performed using the Statistical Package Program PASW® Statistics 18 for Windows. The Shapiro-Wilk test was used as a test of normality to assess the distributional adequacy of the variables. Multiple comparisons were performed by Anova test with Tukey's post hoc test for normally distributed parameters to detect differences among the groups. For the variables that do not exhibit a normal distribution Kruskal Wallis and Mann Whitney tests were used. $p<0.05$ was the treshold for statistical significance.

III. Results

Forty obese, 40 asthmatic, 40 obese asthmatic and 40 healthy children participated in the study. Mean ages were 6.9±2.6 years, 5.0±2.3 years, 6.9±2.3 years and 5.4±2.5 years in these groups, respectively. There was not any statistically significant difference between the ages of the groups. Statistically significant difference ($p\leq0.001$) was detected between BMI values of the groups (Fig. 1). There was no significant difference between incomes and parental educational profiles of the groups.

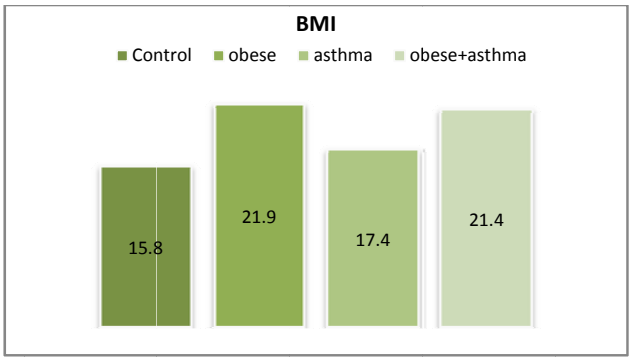

Fig. 1 BMI values of the study groups

A statistically significant difference was found between the waist-to-hip ratios of obese and healthy children ($p\leq0.05$), but not in asthmatic and obese asthmatic children Fig. 2.

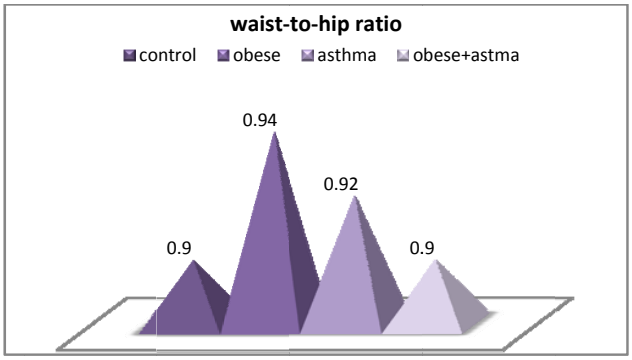

Fig. 2 Waist-to-hip ratios of the study groups

Flow cytometry results demonstrated that the levels of $FoxP3^+$ cells in the peripheral blood of obese, asthmatic and obese asthmatic children were lower than those in normal children, suggesting that Tregs levels were reduced in the peripheral blood during obesity and asthma. Statistically significant decreases were observed for obese ($p\leq0.001$), asthmatic ($p\leq0.01$) and obese asthmatic ($p\leq0.05$) children in comparison with the control group when Tregs were compared. Statistically significant increases in Tregs were observed in asthmatic ($p\leq0.001$) and obese asthmatic ($p\leq0.001$) children compared to those of obese group. There was not any statistically significant difference between Ths % of the control and obese as well as obese asthmatic groups. However, significantly higher Ths were found in asthmatic children in comparison with the control ($p\leq0.01$) and obese ($p\leq0.001$) groups. Comparison of the values for Ths and Tregs found in the study groups is shown in Fig. 3.

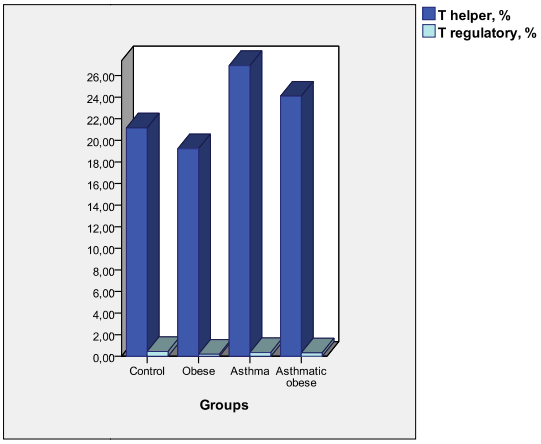

Fig. 3 The values for Ths and Tregs found in the study groups

Any statistically significant difference between HOMA-IR (1.6±1.5 *vs*2.2±1.7) values of the control and obese groups was not observed. However, statistically higher values (3.1±2.2) were recorded in obese asthmatics (Fig. 4).

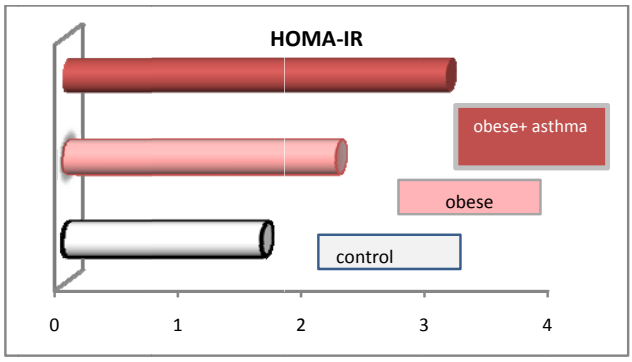

Fig. 4 HOMA-IR values of the study groups

As far as the laboratory findings were considered, any statistically significant difference was not detected for wbc count (n) (7800±2500 *vs* 8600±2800) and neutrophile (%) (41.1±14.1 *vs* 45.3±13.7) between control and obese groups. Similar pattern was observed for wbc count (n) (7800±2500 *vs* 8000±1300) and neutrophile (%) (41.1±14.1 *vs* 46.1±7.9) between control and obese asthmatic groups. Significant increases were noted in asthmatic group for wbc count (7800±2500*vs* 9650±2700; p≤0.005) and total IgE (IU/ml) (56.3±77.9 vs 158.3±222.1; p≤0.01). IgE values were higher (56.3±77.9 vs 155.7±179.6; p≤0.01) also in obese asthmatic group.

IV. Discussion

Tregs mediated suppression mechanisms are numerous and complex. The suppressing roles of Tregs in adipose tissue inflammation have been pointed out [18]. Hyperinsulinemia may impair the ability of Tregs to suppress inflammatory responses and may contribute to the development of obesity-associated inflammation [19]. The production and propagation of Tregs may be promising in alternative asthma and obesity treatments [20]. The determination of circulating Tregs may be clinically useful for distinguishing "healthy" obese subjects from those at increased risk to develop cardiovascular diseases or metabolic complications of increased body weight [21].

To our knowledge, there is not any research on Tregs as well as the expression of Tregs-associated proteins such as FoxP3, CD25 in obese, asthmatic and obese asthmatic children. This study is the first to show the impaired function of circulating Tregs in obese, asthmatic and obese asthmatic children and investigating the status of HOMA-IR and Tregs in obese and obese asthmatic children in comparison with those of the control group children. Obesity, as a low grade inflammatory disease, is closely associated with some chronic diseases including asthma, which are also known with their inflammatory aspects.

Enhanced adipocyte differentiation is associated with inhibition of FoxP3 Tregs expression, thus promotes growth, obesity and allergy development [22]. Aside from the increased BMI values observed in obese and obese asthmatic groups, our study has shown that children with asthma exhibit also higher BMIs than those without asthma. This finding is consistent with the results of other studies [23].

We have detected significantly reduced Tregs in obese, asthmatic and obese asthmatic children in comparison with the healthy children. Significant increases in Tregs were found in asthmatic, and obese asthmatic children compared to those of obese group. Impaired immunosuppression of CD4 T-cell activation mediated by Tregs expressing FoxP3 was detected in all of the patient groups. Lower Tregs with significantly higher FoxP3 expression in children with obesity, asthma and obesity+asthma compared to those in healthy controls may be the indicator of partial suppressive function.

Obesity and asthma are the most common chronic morbidities in children in developing and developed regions. Obesity is higher in children with asthma. Manifestations typical of excess adiposity e.g. IR, may be important in the asthma-obesity link [24].

Our study is one performed on 160 children and upon evaluation of the insulin sensitivity index (ISI); HOMA-IR levels, any significant difference between control and obese groups couldn't be noted, however, significantly increased values were observed for obese asthmatics. It is interesting to note that the values obtained in all groups were found to be below the critical cut off points 2.5 or 3.16. This finding has made the statistically significant differences observed between Tregs of control group and those of obese, as well as obese asthmatics much more valuable. Significantly reduced Tregs were detected in obese, asthmatic and obese asthmatic children compared to those in control group. This finding is the indicator of the fact that Tregs serve as anti-inflammatory immune system cells.

So far, we did not find a study investigating the relationship between the IR status (by HOMA-IR) and Tregs count in pediatric obesity and asthmatic obesity. Considering the association between Tregs and IR; some data showed that obese patients with IR display significantly decreased Tregs,

present evidence that Tregs are key regulatory cells in the pathogenesis of IR and suggest a potential therapeutic value of Tregs to improve IR by limiting the proinflammatory milieu [25]. On the other hand, some other data on pediatric obstructive sleep apnea reported that IR is not associated with circulating Tregs and their suppressive functions and do not support a relationship between Tregs and IR [26]. Our results were consistent with the findings of this study.

The present study investigating the association between HOMA-IR and Tregs in obese and obese asthmatic Turkish children is, to the best of our knowledge, the first to show that the frequency of CD4+CD25+FoxP3+Tregs in peripheral lymphocytes is lower in obese and obese asthmatic children than in controls, where the cohorts were matched for IR. Considering the facts that HOMA-IR is one of the most reliable commonly used IR/ISI index, and the link between obesity and IR, this finding makes the Tregs analysis in obese and obese asthmatic children much more meaningful. This point may emphasize the prognostic value of low Tregs with regard to diabetes development as well as cardiovascular mortality.

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