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Association between Type 2 Diabetes, Obesity and Key Immunological Components of IgE-mediated Inflammation

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Background: Changes in lifestyle and obesity in recent decades have brought about a dramatic increase in type 2 diabetes mellitus (DM2) and allergic diseases. Clinical and epidemiological studies associate obesity with epidemics of allergic diseases. The link between obesity and DM2 with immunological components of IgE-mediated allergic inflammation is not yet conclusively established.

Aim: To examine the key immunological components of IgE-mediated allergic inflammation in patients with DM2 and their relationship with glycemic control and anthropometric indicators.

Materials and methods: Fifty-five patients with DM2 and 32 healthy controls with normal weight and body mass index (BMI) of 18-24.9 kg/m^2 were included in the study. Th2-cytokine profile (serum levels of IL-4 and IL-5, pg/ml) and total serum IgE IU/ml were assessed in all participants in the study using ELISA. In patients with DM2, levels of glycated hemoglobin (HbA1c%) in the blood were also measured.

Results: Serum levels of IL-4 and IL-5 are significantly higher in patients with DM2 compared to the control group. Serum levels of IL-4 and IL-5 positively correlated with BMI as well as serum levels of IL-4 with waist circumference. Total serum IgE positively correlated with HbA1c.

Conclusion: Obesity and poor glycemic control in patients with DM2 affect key immunological components of IgE-mediated allergic inflammation and possibly alter the immune response to allergens and antigens.

BACKGROUND

DM2 is a metabolic disorder characterized by hyperglycemia, insulin resistance and relative lack of insulin.[1] The increase of the number of patients with DM2 worldwide is associated with the epidemic of obesity.[2] Clinical and epidemiological studies have found association between obesity and increasing incidence of allergic diseases in last decade.[3-5] Obesity probably induces changes in the immune response concerning disorders in Th1/Th2 immune profile by skewing of the immune system towards Th2.[6] Changes in the serum levels of Th2 cytokines IL-4 and IL-5 and total IgE are not sufficiently studied in patients with DM2. In this study we investigated the Th2 cytokine profile and total IgE in patients with DM2 and healthy controls with normal weight, and their relationship with glycemic control and anthropometric parameters.

MATERIALS AND METHODS

Study population

Fifty-five patients with DM2 and 32 healthy controls with normal BMI (18-24.9 kg/m^2) were included in the study. The patients with DM2 were previously diagnosed with DM2 and duration of illness > 6

months. The study received the approval of the Medical University, Plovdiv Ethics Committee and obtained prior written informed consent of patients with DM2 and those of the control group. We met all requirements of the Helsinki Declaration of Patients Rights. Studies were carried out according to the requirements of Good Clinical Practice (Good Clinical Practice). The demographic data are presented in **Table 1**.

rank correlation coefficients (r_s) was used. All analyses were performed using SPSS v. 20.0. $P < 0.05$ was assumed for the level of statistical significance.

RESULTS

Table 1 presents the demographics and the anthropometric parameters of the studied contingent, and the mean HbA1c% levels in DM2 patients. The mean age of patients with DM2 was significantly higher than that of the control group due to difficulties in recruiting healthy controls with BMI of 18-24.9 kg/m^2 comparable to the age of these patients. The mean levels of HbA1c in DM2 patients were 7.789 ± 1.169%, which indicates that most of them had poor glycemic control. **Table 2** shows the serum levels of IL-4, IL-5 and total IgE antibodies in DM2 patients and in the controls. IL-4 serum levels were significantly higher in patients with DM2 (13.128 ± 15.162 pg/ml) compared to those in the controls (10.239 ± 3.524 pg/ml, p = 0.000). Likewise, the serum levels of IL-5 were significantly higher in patients with DM2 (9.745 ± 1.258 pg/ml) than those in the controls (8.962 ± 1.388 pg/ml, p = 0.036). There were no significant differences in the serum levels of total IgE between patients with DM2 (138.657 ± 153.136 IU/ml) and controls (78.542 ± 70.204 IU/ml, p = 0.341). For comparison of Th2 cytokines and total IgE between the two groups, we used non-parametric Mann-Whitney U test.

Table 1. Demographic and anthropometric parameters, and HbA1c in patients with DM2

Parameters	Diabetic patients Mean ± SD*	Non-diabetic healthy controls Mean ± SD	p-value**
Number (n)	55	32	
Age (years)	59.15±8.225	50.34±16.122	0.032
BMI (kg/m^2)	34.224±7.026	22.130±1.876	0.000
Waist circumference (cm)	108.31±14.765	76.16±7.314	0.000
HbA1c (%)	7.789±1.169		

*Mean and standard deviation, **p<0.05 is with statistical significance

Anthropometric parameters and glycemic control

We measured height and weight and calculated BMI. BMI was calculated using the standard formula for each participant in the study [BMI = weight (kg)/ height^2 (m^2)]. Waist circumference (cm) was measured in mid-axillary line connecting the lower edge of the 12th rib and the top of the iliac crystals. In patients with DM2, glycated hemoglobin (HbA1c%) was measured by immune-inhibition test of venous whole blood TINIA (turbidimetric inhibition immunoassay for hemolyzed whole blood).

Th2 cytokine profile (IL-4 and IL-5) and total IgE antibodies

Patients' sera were used after separation from the blood by centrifugation for 5 minutes. ELISA Human IL-4 Platinum, ELISA Human IL-5 Platinum kits (eBioscienceDx Diagnostics) and ELISA Total IgE kit (EUROIMMUN Germany) were used to measure serum IL-4, IL-5 and total IgE levels.

Statistical analysis

Quantitative variables were tested for normality of the distribution by the Shapiro-Wilk test. Mann-Whitney U test was used to compare quantitative variables. To investigate the relationship between quantitative variables and misallocation, Spearman

Tables 3 and **4** present the correlations between Th2 cytokines and anthropometric parameters. We found a positive, statistically significant correlation between serum levels of IL-4 and BMI (r_s = 0.277, p = 0.009). Positive, statistically significant correlation was found between the serum levels of IL-4 and the waist circumference (r_s = 0.238, p =

0.027). Positive, statistically significant correlation was also found between serum levels of IL-5 and BMI (r_s = 0.245, p = 0.025). The serum levels of IL-5 did not correlate with waist circumference (r_s = 0.086, p = 0.439). We found a positive, statistically significant correlation between HbA1c and serum total IgE (r_s = 0.298, p = 0.027).

Table 2. Comparison between serum levels of IL-4 pg/ml, IL-5 pg/ml and total IgE IU/ml between patients with DM2 and control group without DM2 with BMI of 18-24.9 kg/m^2

Parameters	Diabetic patients (Mean ± SD)*	Non-diabetic healthy controls (Mean ± SD)	p-value**
Number (N)	55	32	
IL-4 pg/ml	13.128±15.162	10.239±3.524	0.000
IL-5 pg/ml	9.745±1.258	8.962±1.388	0.036
IgE IU/ml	138.657±153.136	78.542±70.204	0.341

*Mean and standard deviation, **p<0.05 is with statistical significance

Table 3. Relationship between anthropometric measurements and serum levels of IL-4

Cytokines and anthropometric measurements	r_s*	p-value**
IL-4 (pg/ml)	0.277	0.009
BMI (kg/m^2)	0.277	0.009
Waist circumference (cm)	0.238	0.027

*Spearman rank correlation coefficients (r_s), **p<0.05 is with statistical significance

Table 4. Relationship between anthropometric measurements and serum levels of IL-5

Cytokines and anthropometric measurements	r_{s*}	p-value**
IL-5 (pg/ml)	0.245	0.025
BMI (kg/m^2)	0.245	0.025
Waist circumference (cm)	0.086	0.439

*Spearman rank correlation coefficients (r_s), **p<0.05 is with statistical significance

DISCUSSION

We found that the serum levels of Th2 cytokines IL-4 and IL-5 were significantly higher in patients with DM2 than in healthy controls without DM2 with BMI of 18 - 24.9 kg/m^2. These data are in concordance with the data reported by other researchers. Anand G et al. also found high serum levels of the Th2 cytokines IL-4 and IL-13 in patients with DM2 compared with the control group of individuals without DM2 and DM2 patients with diabetic nephropathy. Moreover, they observed a mixed Th1/Th2 cytokine profile in patients with DM2.[7] Dezayee ZM also observed higher serum levels of IL-4 in patients with DM2 compared to the control group.[8] IL-4 is one of the key cytokines involved in allergic inflammation, whose primary role is to stimulate the synthesis of IgE antibodies. The main sources of IL-4 are Th2 lymphocytes, mast cells, eosinophils, basophils and NK cells.[9-12] Adipocytes are also a source of Th2 cytokines, IL-13 and, to a lesser extent, IL-4.[13] Besides the role it plays in IgE-mediated inflammation, IL-4 is involved in the metabolic control by improving insulin sensitivity and glucose tolerance.[14] The pathophysiological and immunological mechanisms that lead to increased

serum levels of IL-4 and participation in DM2, metabolic control and insulin resistance are still a mystery. We found a positive correlation between serum levels of IL-4 with BMI and IL-4 in waist circumference. In previous studies we found that BMI positively correlated with waist circumference (data not shown). The increase in adipose tissue with the development of low-grade inflammation and the production of a wide range of adipokines associated with obesity, insulin resistance and DM2 is quite likely to affect the immune response by stimulating the release of Th2 cytokines. El-Wakkad A et al. found a positive correlation between serum levels of IL-4 with BMI and the waist-to-hip ratio in female adolescents.[15] In addition, they observed a positive correlation between serum levels of key adipokines leptin and IL-4. The relationship between leptin and Th2 cytokines is not fully understood. Increased circulating levels of leptin are a marker of leptin resistance.[16] Literature data show that leptin resistance in obesity may alter the polarization of the immune response to Th2.[17] On the other hand, some studies have observed a positive correlation between serum IL-4 and plasma glucose.[18] In our study a correlation between HbA1c and serum IL-4 was not observed.

IL-5 is another Th2 cytokine with a key role in allergic inflammation. IL-5 is a primary activator of eosinophils and a chemoattractant.[19] We found that serum levels of IL-5 were higher in patients with DM2 compared to the control group. Lucas R et al. observed significantly higher levels of IL-5 in plasma in patients with pre-diabetes.[20] On the one hand, Ahmad R et al.[21] have demonstrated that patients with DM2 and obesity have significantly higher levels of IL-5, as compared to patients with DM2, overweight and BMI of 18-25 kg/cm^2. They found a correlation between IL-5 and BMI.[21] We also found a positive correlation between serum levels of IL-5 with BMI. On the other hand, Ahmad R et al. found a positive correlation between IL-5 and HbA1c% in patients with DM2.[21] We did not find any correlation between IL-5 and HbA1c.

IgE Abs play a key role in IgE-mediated hypersensitivity reactions. IgE antibodies bind to the Fc region of the molecule with Fc-receptors located on the surface of cells involved in IgE-mediated allergic immune response. Fixing the allergen with two adjacent molecules of IgE associated with mast cells is a trigger of an allergic reaction.[22] Wang Z et al. observed that the serum levels of IgE were significantly higher in patients with diabetes and pre-diabetes in comparison with normal glycemic persons.[23,24] We have found no significant difference in the present study in the serum levels of total IgE between patients with DM2 compared to the control group. We have found a correlation between serum IgE levels and HbA1c. Heshmatolah P et al. associated the higher serum levels of IgE antibodies in patients with DM2 with the HbA1c and blood glucose concentration.[25] Wang Z et al. have also established the association between serum levels of IgE antibodies with glucose status.[23]

Metabolic factors influencing the immunological components of IgE-mediated inflammation in patients with DM2 are quite different.[23-25] Obesity is the main link between DM2 and IgE-mediated allergic inflammation.[4,5]

CONCLUSION

With the increase of BMI and waist circumference there are changes of cytokine profile towards Th2 phenotype shifting. On the other hand poor glycemic control presumably leads to increased IgE antibodies. All these changes in the immunological components of IgE-mediated inflammation may reduce the immune tolerance to allergens. Further studies are needed to determine the risk of IgE-mediated allergic diseases in patients with DM2 in association with obesity.

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Связь между диабетом 2 типа, ожирением и ключевыми иммунологическими компонентами иммуноглобулин Е - опосредованного воспаления

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Введение: Изменения в образе жизни и ожирение за последние десятилетия вызвали резкий рост заболеваемости сахарным дибетом 2 типа (СД2) и аллергических заболеваний. Клинические и эпидемиологические исследования устанавливают связь между ожирением и эпидемией аллергических заболеваний. Связь между ожирением и СД 2 с иммунологическими компонентами IgE-опосредованного аллергического воспаления не является бесспорно установленной.

Цель: Целью данной работы являлось исследование ключевых иммунологических компонентов IgE – опосредованного аллергического воспаления у пациентов с СД 2 и их взаимосвязь с гликемическим контролем и антропометрическими показателями.

Материалы и методы: Пятьдесят пять пациентов с СД 2 и 32 здоровых участника в качестве контрольной группы с нормальным весом и индексом телесной массы (ИТМ) 18-24.9 кг/м2 приняли участие в исследовании. Th2-цитокиновый профиль (сывороточные уровни интерлейкина (IL-4) и интерлейкина 5 (IL-5), пгр/мл) и общий сывороточный IgE IU/ml были установлены при помощи ELISA у всех пациентов исследования. У пациентов с СД 2 также были измерены уровни гликозилированного гемоглобина (HbA1c%) в крови.

Результаты: Сывороточные уровни IL-4 и IL-5 оказализь в значительной степени более высокими у пациентов с СД 2 по сравнению с контрольной группой. Сывороточные уровни IL-4 и IL-5 коррелируют положительно с ИТМ, как и сывороточные уровни IL-4 с окружностью талии. Общий сывороточный IgE коррелирует положительно с HbA1c.

Заключение: Ожирение и неудовлетворительный гликемический контроль у пациентов с СД 2 затрагивают ключевые иммунологические компоненты IgE-опосредованного аллергического воспаления и по-видимому приводят к изменению иммунного ответа на аллергены и антигены.