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Trabajo Original

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Determination of inflammation by TNF-alpha and IL-10 levels in obese children and adolescents

Determinación de la inflamación mediante los niveles de TNF-alfa e IL-10 en niños y adolescentes con obesidad

Ali Gul[1], Resul Yilmaz[2]

[1]Department of Pediatrics. Gaziosmanpasa University School of Medicine. Tokat, Turkey. [2]Department of Pediatrics. Division of Pediatric Intensive Care Unit. Selcuklu University School of Medicine. Konya, Turkey

Abstract

Background: childhood obesity is one of the major health problem worldwide. Obesity is associated with low-level chronic inflammation resulting from inflammatory cytokine release in white adipose tissue. We aim to specify inflammatory markers tumor necrosis factor-alpha (TNF-alpha) and interleukin-10 (IL-10) in children and adolescents to determine their relationship with obesity.

Materials and methods: forty obese patients and 46 controls were included in the study from the pediatric clinic. Blood samples from the study group were centrifuged, and the sera were stored at -80 °C after separation. Serum levels of TNF-alpha and IL-10 were determined using Human ELISA kits for TNF-alpha and IL-10.

Results: serum samples from 86 children, including 45 girls (52.3 %) in the study group, were analyzed for TNF-alpha and IL-10 levels. TNF-alpha levels in the obese and control groups were 1.04 ± 0.79 and 0.60 ± 0.72 pg/ml, respectively ($p = 0.010$). Also, IL-10 levels in the obese and control groups were 0.76 ± 0.62 and 1.54 ± 0.71 pg/ml, respectively ($p < 0.001$). Gender was not identified as a factor for serum TNF-alpha and IL-10 levels ($p = 0.281$ and $p = 0.477$, respectively). Moreover, white blood cell (WBC) and serum C-reactive protein (CRP) levels were higher in the obese patient group than in the control group ($p = 0.002$ and $p = 0.010$, respectively).

Conclusion: TNF-alpha levels were higher than control in obese patients and it was important in terms of showing that obesity triggers inflammation in the body. IL-10 levels, which inhibit inflammation, were lower in obese patients than controls.

Keywords:

Obesity. Child. TNF-alpha. Interleukin-10. Inflammation. Cytokine.

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Correspondence:

Ali Gul. Department of Pediatrics. Gaziosmanpasa Universitesi TIP Fakultesi Pediatri AD 60030 Tokat/ Turkey
e-mail: draligul@yahoo.com

Resumen

Antecedentes: la obesidad infantil es uno de los principales problemas de salud a nivel mundial. La obesidad está asociada con una inflamación crónica de bajo nivel, resultado de la liberación de citocinas inflamatorias en el tejido adiposo blanco. Nuestro objetivo es especificar los marcadores inflamatorios factor de necrosis tumoral-alfa (TNF-alfa) e interleucina-10 (IL-10) en niños y adolescentes para determinar su relación con la obesidad.

Materiales y métodos: cuarenta pacientes obesos y 46 controles fueron incluidos en el estudio desde la clínica pediátrica. Las muestras de sangre del grupo de estudio se centrifugaron, y los sueros se almacenaron a -80 °C después de la separación. Los niveles séricos de TNF-alfa e IL-10 se determinaron utilizando *kits* ELISA humanos para TNF-alfa e IL-10.

Resultados: se analizaron muestras de suero de 86 niños, incluidas 45 niñas (52,3 %) en el grupo de estudio, para los niveles de TNF-alfa e IL-10. Los niveles de TNF-alfa en los grupos de obesos y control fueron de 1,04 ± 0,79 y 0,60 ± 0,72 pg/ml, respectivamente ($p = 0,010$). Además, los niveles de IL-10 en los grupos de obesos y control fueron de 0,76 ± 0,62 y 1,54 ± 0,71 pg/ml, respectivamente ($p < 0,001$). El género no se identificó como un factor para los niveles séricos de TNF-alfa e IL-10 ($p = 0,281$ y $p = 0,477$, respectivamente). Además, los niveles de glóbulos blancos (WBC) y proteína C-reactiva (PCR) en suero fueron más altos en el grupo de pacientes obesos que en el grupo de control ($p = 0,002$ y $p = 0,010$, respectivamente).

Conclusión: los niveles de TNF-alfa fueron más altos en el grupo de pacientes obesos que en el grupo control lo que es importante para mostrar que la obesidad desencadena inflamación en el organismo. Los niveles de IL-10, que inhiben la inflamación, fueron más bajos en pacientes obesos que en controles.

Palabras clave:

Obesidad. Niño. TNF-alfa. Interleucina-10. Inflamación. Citocina.

INTRODUCTION

Obesity has become a significant global health problem due to its strong association with diseases such as insulin resistance, type 2 diabetes, atherosclerosis, and ischemic heart disease, which reduce life expectancy and have significant economic and societal impacts. Obesity is associated with inflammatory cytokine (IC) release in white adipose tissue (AT), triggering local inflammation, as well as with low-grade chronic inflammation in adipocytes (1,2). Furthermore, the pathogenesis of obesity-related metabolic disorders is known to play a crucial role in chronic inflammation. Low-grade inflammation has been clearly linked to metabolic disorders such as type 2 diabetes *mellitus* (DM). Increased AT mass leads to elevated pro-inflammatory markers such as C-reactive protein (CRP) and its inducer, interleukin (IL)-6. Interestingly, elevations in CRP and IL-6 were found to predict the development of type 2 DM (3,4). AT also causes the production of inflammatory mediators known as adipokines; when released into the circulation, these transport the local inflammation caused by obesity to other parts of the body, including cardiovascular tissues (5).

In obesity and diabetes, tumor necrosis factor-alpha (TNF-α) has been identified as a pro-inflammatory product of AT which links obesity and inflammation (6). A previous study revealed that mice with inactivated insulin receptors are protected from obesity-related insulin resistance and inflammation (1). This shows that in obesity, the insulin receptor plays a key role in macrophage activation and inflammation (2). Accordingly, obese children also had increased circulating levels of pro-inflammatory cytokines (e.g., leptin, TNF-α, IL-6, and CRP) compared to normal-weight children and adolescents aged 2-18 years (3). Specifically, TNF-α activates macrophages in host defense mechanisms, inducing the production of pro-inflammatory nitric oxide and reactive oxygen species (7).

IL-10 has been recognized as an anti-inflammatory and immunosuppressive factor. As an anti-IC, IL-10 suppresses the ability of human monocytes and macrophages to produce pro-ICs, including IL-6 (8). IL-10 is produced by activated immune cells (T and B lymphocytes, mast cells, granulocytes, macrophages) which acts as a potent negative feedback regulator and controls inflammation (7). Within lean AT, anti-ICs secreted by resident AT macrophages (ATMs) help maintain insulin sensitivity by counteracting inflammatory responses. Indeed, treatment of adipocytes with IL-10 alleviated the insulin resistance (IR) induced by TNF-α (9).

To the best of our knowledge, no study has evaluated the role of IL-10 in childhood obesity. This study aimed to identify pro-inflammatory and anti-inflammatory markers present in children and adolescents to determine their relationship with obesity.

MATERIALS AND METHODS

Children and adolescents (40 obese, 46 controls) were recruited from the pediatric outpatient department of the hospital of Tokat Gaziosmanpasa University School of Medicine, Tokat, Turkey. The study was conducted in accordance with the Helsinki Declaration of the World Medical Association and local ethical standards; this was approved by the Ethics Committee of Gaziosmanpasa University School of Medicine (approval no. 17-KAEK-014). Since can cause inflammation, subjects with symptoms or signs of infection, fever, or/and endocrinologic disorders such as thyroiditis or rheumatologic diseases such as arthritis or rash were excluded. The study group was classified according to the age and sex-adjusted body mass index (BMI) reference curve for Turkish children. Participants with BMI above the 95th percentile were classified as obese, while those between the 5th and 84th percentiles as normal (10) (Table I).

Serum samples were obtained from fasting blood samples and stored at -80 °C. TNF-α and IL-10 were measured using an ELISA kit (Elabscience Biotechnology Co., Wuhan, China). Laboratory tests, including complete blood count, glucose, blood urea nitrogen, serum creatinine (sCre), CRP, aspartate transaminase (AST), and alanine transaminase (ALT), were obtained from fasting blood samples of all participants. Insulin levels and thyroid function tests were measured in obese participants only. Serum fasting glucose, insulin, AST, ALT, and sCre were measured using reagent kits from Roche Diagnostics adapted to the COBAS

Table I. Distribution of the study group and inflammatory markers according to gender

		Group		*p*	Inflammatory marker		*p*
		Obese *n* (%)	Control *n* (%)		TNF-α (pg/mL)	IL-10 (pg/mL)	
Sex	Female	24 (60.0)	21 (45.7)	0.184	0.89 ± 0.84	1.12 ± 0.83	0.281
	Male	16 (40.0)	25 (54.3)		0.71 ± 0.72	1.24 ± 0.70	0.477
Total		40 (100)	46 (100)		0.81 ± 0.78	1.17 ± 0.77	

E601 system (Roche Diagnostics, Mannheim, Germany). Statistical analysis of the data was performed using SPSS 19 (IBM SPSS Statistics 19, SPSS Inc., Somers, NY, USA), and $p < 0.05$ was considered significant.

RESULTS

Serum TNF-α and IL-10 levels were measured in 40 obese children and 46 controls, who had mean ages of 12.7 ± 3.2 and 13.1 ± 2.3 years, respectively ($p = 0.698$). Among the participants, 53.5 % were female. When grouped according to sex, there were no significant differences in TNF-α and IL-10 levels. TNF-α levels were 0.892 ± 0.835 and 0.708 ± 0.716 pg/mL in females and males, respectively ($p = 0.281$). IL-10 levels were 1.117 ± 0.832 and 1.238 ± 0.705 pg/mL in females and males, respectively ($p = 0.477$) (Table II).

Serum TNF-α levels were significantly higher in obese individuals versus controls (1.04 ± 0.79 vs. 0.60 ± 0.72 pg/mL, $p = 0.010$) IL-10, known for its anti-inflammatory properties, was lower in obese individuals versus controls (0.76 ± 0.62 vs. 1.54 ± 0.71 pg/mL, $p < 0.001$).

Table II. Distribution of TNF-α and IL-10 serum levels and laboratory parameters by groups

Variables	Group			*p*
	Total (*n* = 86)	Control (*n* = 46)	Obese (*n* = 40)	
	Mean ± SD	Mean ± SD	Mean ± SD	
IL-10 (pg/ml)	1.174 ± 0.773	1.545 ± 0.709	0.756 ± 0.618	*< 0.001*
TNF-α (pg/ml)	0.806 ± 0.782	0.602 ± 0.722	1.035 ± 0.792	*0.010*
Age (year)	12.8 ± 3.0	12.7 ± 3.2	13.1 ± 2.3	0.698
Height (cm)	152.86 ± 14.05	151.41 ± 17.24	154.52 ± 9.04	0,672*
Body weight (kg)	54.25 ± 18.24	47.36 ± 15.28	62.18 ± 18.32	0,138*
BMI (kg/m^2)	22.77 ± 5.95	20.33 ± 5.72	25.58 ± 4.92	0,342*
TSH	2.437 ± 1.299	2.311 ± 1.18	2.711 ± 1.53	0.298
fT4	1.206 ± 0.583	1.126 ± 0.486	1.408 ± 0.756	0.092
Glucose	90.49 ± 9.82	91.69 ± 10.87	87.72 ± 6.27	0.166
Insulin	24 ± 18.01	20.29 ± 15.83	24.87 ± 18.82	0.659
Hemoglobin	12.99 ± 1.34	12.99 ± 1.42	12.98 ± 1.12	0.973
White blood cells	7.25 ± 2.1	6.79 ± 1.82	8.6 ± 2.35	0.002
Platelets	298.87 ± 66.33	284.93 ± 58.66	341.6 ± 72.13	0.003
CRP	6.69 ± 24.52	2.69 ± 5.04	30.01 ± 62.24	0.010
AST	21.55 ± 6.39	21.43 ± 6.58	21.88 ± 6.01	0.809
ALT	16.79 ± 10.48	15.48 ± 7.51	20.32 ± 15.77	0.104

*TNF-α: tumor necrosis factor-alpha; IL-10: interleukin-10; BMI: body mass index; TSH: thyroid stimulating hormone; fT4: serum free thyroxin; CRP: C-reactive protein; AST: aspartate transaminase; ALT: alanine transaminase. The values presented are means ± standard deviation (SD). The p-values represent the statistical significance for the comparison between the control and obese groups. *Correction for age and gender was performed by covariance analysis.*

DISCUSSION

Chronic inflammation, as seen in obesity, can occur due to excess production of inflammatory mediators when clearance mechanisms are insufficient or even under normal physiological changes. Circulating inflammatory mediators and activated monocytes have been associated with metabolic and cardiovascular complications in obesity (5). In this study, we aimed to assess inflammation by determining the levels of inflammatory markers TNF-α and IL-10. Notably, TNF-α, which triggers inflammation by promoting macrophage activation and oxygen radical production (8), was higher in obese children and adolescents. IL-10, which has anti-IC actions and suppresses monocyte/macrophage stimulation and cytokine production, especially IL-6 (7), was lower in obese children and adolescents.

Previous studies have associated subclinical chronic inflammation with obesity (11). AT acts not only as a fat depot but also as an active endocrine organ which releases numerous peptides and cytokines into the circulation (12). In obesity, there is a shift in the balance among these molecules; enlarged adipocytes produce more pro-ICs (e.g., TNF-α and IL-6) and fewer anti-inflammatory peptides (e.g., adiponectin) (13). The infiltration of macrophages into AT was also found to increase with the severity of obesity, which is associated with decreased insulin sensitivity (14). The dysregulation of these adipokines reportedly plays a significant role in obesity-related metabolic and cardiovascular disorders (15). Severely obese adolescents with increased BMI and CRP have an increased risk of impaired fasting glucose and hypertension (16). Similarly, regardless of the association with metabolic complications, we also found elevated TNF-α levels in obese children and adolescents. Kopp et al. reported that TNF-α levels decreased alongside weight loss in obese patients with metabolic syndrome (17). Thus, weight loss can possibly achieve inflammation control in obesity-related metabolic disorders.

Protective factors, such as adiponectin, reportedly decrease in cases of increased BMI and elevated CRP (18). IL-10 is considered a good and protective cytokine in human metabolism (19). In atherosclerosis, IL-10 is speculated to be produced in the atherosclerotic plaques by stimulated monocytes/macrophages and lymphocytes, and it may provide more protection from excessive pro-inflammatory responses (20). Jung et al. showed that an increase in IL-10 was dependent on the degree of weight loss and improvement in metabolic disorders (21). In our study, IL-10 levels were low in obese adolescents and children. Since we did not observe the effects of weight loss, we cannot make interpretations about the changes in this cytokine. In contrast, it has been suggested that IL-10 release is related to TNF-α and could help limit the pro-inflammatory effect of TNF-α (21).

These studies have shown that low-grade systemic inflammation is present in childhood obesity. Consequently, it has been proposed that metabolic dysfunction associated with excessive AT mass could result from an imbalance in the expression of pro- and anti-inflammatory adipokines, thereby contributing to the development of obesity-related complications (5,22).

A limitation of this study is the lack of investigation into the relationship between metabolic disorders and inflammatory markers. Nevertheless, as a case-control study, the clear differences in these markers highlight the importance of our findings.

In conclusion, this study examined two inflammatory markers in childhood obesity, TNF-α and IL-10. Inflammatory mediators differed between obese and lean control groups. These ICs may be part of the inflammatory connection between obesity and its metabolic and cardiovascular complications. Thus, these can be potential targets for the early detection and prevention of these complications.

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