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ORIGINAL ARTICLE

Proinflammatory state in obese children

Estado Proinflamatorio en niños obesos

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Abstract

Introduction: Obesity is a chronic disease that affects adults as well as children and is associated with insulin resistance, type 2 diabetes and cardiovascular disease. One of the reasons for the presence of low-grade inflammation in these patients could be that adipose tissue of the obese produces proinflammatory molecules that favor the development of atherosclerosis. **Objective:** To determine serum levels of soluble CD40 ligand (sCD40L), monocyte chemoattractant protein 1 (MCP-1), interleukin 6 (IL-6), Tumor Necrosis Factor alpha (TNF-α) and high sensitivity CRP (hsCRP), in an obese children population compared to a control group, also to analyze the correlation of these molecules with the anthropometric and metabolic variables. **Patients and Method:** A cross-sectional, observational study was carried out on 37 obese children, aged 8 to 12 years, and 20 children with normal weight. Serum levels of sCD40L, MCP-1, IL-6, TNF-α and hsCRP were determined. Data were expressed as the median and interquartil range and Spearman coefficient was used to investigate correlations between variables. **Results**: Compared to the control group, obese children presented significantly higher values of sCD40L, MCP-1, IL-6, TNF-α, and hsCRP than control group. Body mass index and waist circumference correlated positively with sCD40L and MCP-1. **Conclusion**: Elevated levels of the studied molecules studied suggest the presence of low-grade inflammation associated with obesity in this population.

Keywords:
Obesity;
subclinical inflammation;
TNF-α;
hsPCR

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Introduction

Obesity is a metabolic and inflammatory disease that has become an epidemic, not only in adults but also in children and adolescents. Obesity prevalence has increased in recent decades and it is considered the most common chronic non-communicable disease in the world[1]. Childhood obesity often continues into adulthood and is closely associated with insulin resistance (IR), dyslipidemia, high blood pressure, and type 2 diabetes[2].

Several processes are involved in the association between obesity and inflammation, including adipocyte hypertrophy and hyperplasia, macrophage recruitment, increased hypoxia, increased oxidative stress, and increased secretion of inflammatory cytokines such as Tumor Necrosis Factor α (TNF-α), Interleukin 6 (IL-6), and Monocyte Chemoattractant Protein-1 (MCP-1)[3,4]. Cytokines have a profound effect on insulin sensitivity in the liver, peripheral tissues, and vascular endothelial homeostasis[5].

However, as in many pathophysiological situations, childhood obesity has its own characteristics that would include the synthesis and secretion of adipokines. Thus, childhood and adolescence are periods of intense adipogenesis and the accumulation of fatty tissue would be associated mainly with the increase in the number of normal-sized adipocytes (hyperplastic obesity). In addition, the adipokines secretion pattern would depend on the size of the adipocytes[6,7].

TNF-α, produced by adipose tissue, macrophages, and endothelial cells, participates in several physiological processes in response to injuries, infections, angiogenesis and/or apoptosis. The TNF-α has an important role in insulin resistance since it inhibits the action of insulin in adipocytes through insulin signaling pathway inhibitors and also appears to be related to peripheral insulin resistance[8]. It also has a damaging effect on vascular homeostasis, through different mechanisms such as the reduction of vasodilatation due to lower bioavailability of nitric oxide (NO), stimulation of adhesion molecules expression in endothelial and smooth muscle cells, and increased apoptosis of endothelial cells[10].

IL-6, also a pleiotropic cytokine, is produced by numerous cells of the immune system, omental and subcutaneous adipose tissue. IL-6 levels are related to body mass index (BMI) and IR. IL-6 is the main regulator of the acute inflammatory response and plays a critical role in chronic inflammation by stimulating the synthesis of C-reactive protein (CRP)[11,12].

Mild elevations of CRP can occur chronically, providing a relatively stable indicator of low-grade inflammation over months or years[13,14]. Several studies showed that BMI and body fat distribution have a strong influence on serum CRP levels[15,16].

MCP-1 and its receptors are crucial in the development of the inflammatory response and in the recruitment of immune cells to sites of inflammation. Elevated levels of MCP-1 have been associated with atherosclerosis and vascular complications related to obesity[17]. On the other hand, the glycoprotein CD40, a ligand member of the TNFs superfamily (CD40L), binds to its specific CD40 receptor on the surface of cells. Both the CD40L and its receptor are expressed in platelets, lymphocytes and a wide variety of cells. The CD40L/CD40 system is involved in the pathophysiology of chronic inflammatory diseases including atherosclerosis and thrombosis[18,19].

Research studies of these molecules in obese Latin American children are limited and the global role of these pathways poorly understood[20]. Therefore, the objectives of this study are to investi1gate the presence of a pro-inflammatory state, through the determination of TNF-α, IL-6, MCP-1, sCD40L and us-CRP serum levels, and to analyze the correlation of these molecules with anthropometric and metabolic variables in a population of obese children.

Patients and Method

Studied population

This observational, analytical and cross-sectional study included 37 children aged 8 to 12 years (18 boys/19 girls) who had consulted the Endocrinology Department of the Hospital del Niño Jesús in Tucuman, Argentina during the period 2013-2015 due to either overweight or obesity. A group of 20 healthy individuals (ten boys/ten girls) of similar gender and age, eutrophics, from a public school in this city were used as a control. Children from both groups who, at the time of the study, had infectious, inflammatory and endocrine diseases, and were in anti-inflammatory treatment were excluded. A conventional serum CRP value higher than or equal to 6 mg/L was used to rule out acute inflammation.

This study was conducted with the approval of the National University of Tucuman, School of Medicine Bioethics Committee, and the signing of the patient informed consent form by the parents and/or patients guardians.

Anthropometric measurements and pubertal stage

Anamnesis was collected from each patient, considering age, weight, height, BMI, waist circumference (WC), Tanner stages, and disease family history. Weight was measured with a beam-balance scale, light clothing, and no shoes. Height was measured with a

stadiometer without shoes heels together, relaxed shouldersand, both arms at the sides of the body. BMI was calculated using the Quetelet index (weight/height^2). Normal weight was defined as BMI between 15 and 84 percentile; overweight between 85 and 97 percentile, and obesity > 97 percentile for age and gender. Percentiles were calculated using the reference tables recommended by the World Health Organization in 2007[21]. WC was measured at navel level, with a non-distensible tape measure. Abdominal obesity was considered with a WC ≥ at 90 percentile for age and gender, according to percentile table from Fernandez et al[22].

Pubertal stage of children and adolescents was determined by the endocrinologist, according to primary and secondary sexual characteristics under Tanner standards. Children with stage Tanner 1 were considered prepubertal and those with Tanner 2 to 5 were considered pubertal[23,24].

The presence of obesity, diabetes and/or cardiovascular disease family history was also checked in the anamnesis interview conducted with patient's parents or guardians.

Table 1. Clinic and metabolic characterístics od the groups studied

	Controls	Obese	p
n	20	37	---
Sex (male/female)	12/8	18/19	0.53*
Prepúberes/Púberes	8/12	18/19	0.73*
Age (years)	10 (9 -12)	10 (8-12)	0.81
BMI (kg/m^2)	18.6 (17.8-19.9)	28.0 (26.2-30.9)	0.0001
Waist circumference (cm)	67 (59-79)	93 (86-103)	0.0001
Blood glucose (mg/dl)	78 (69-83)	75 (68-84)	0.72
Insulin (uUI/ml)	5.5 (4.2-9.0)	18.2 (12.9-30.5)	0.0001
HOMA	1.2 (0.87-2.0)	3.3 (2.4-6.2)	0.001
Total Colesterol (mg/dl)	160 (134-177)	165 (143-196)	0.38
HDL Colesterol (mg/dl)	40 (35-48)	40 (35-44)	0.63
LDL Colesterol (mg/dl)	94 (73-131)	109 (76-138)	0.60
Triglycerides (mg/dl)	69 (61-84)	96 (77-153)	0.01

Chi-square (*) and Mann-Whitney tests were used. Results are expressed as median (with 25th and 75th quartiles), being significant $p < 0.05$.

Table 2. Levels of proinflammatory molecules in obese and controls

	Controles	Obesos	p
TNF-α (pg/ml)	13.0 (11.5-14.7)	15.4 (13.2-24.0)	0.03
IL-6 (pg/ml)	0.6 (0.5-0.8)	1.0 (0.6-2.1)	0.02
MCP-1 (pg/ml)	95 (77-110)	170 (120-200)	0.001
sCD40L (pg/ml)	112 (90-126)	945 (758-1120)	0.0001
hsPCR (mg/l)	0.3 (0.2-0.8)	1.9 (0.8-3.1)	0.001

Mann-Whitney test were used. Results are expressed as median (with 25th and 75th quartiles), being significant $p < 0.05$.

Biochemical determinations

After a 12-hour fast, a blood sample was obtained and the serum was analyzed for TNF-α, IL-6, MCP-1 (ELISA method, R&D Systems, USA), sCD40L (ELISA method, PeproTech, USA), ultra-sensitive CRP, (us-CRP chemiluminescence method, Immunolite 2000, Siemens), fasting blood glucose (colorimetric method, Wiener Lab, Argentina), plasma insulin (ECLIA method, Roche), and lipid profile (colorimetric method, Wiener Lab, Argentina). The HOMA index was calculated using the Matthews formula and HOMA-I, ≥ 90 percentile was considered IR, according to gender and Tanner stage, using the percentile table from García Cuartero[25].

Statistical analysis

Data were analyzed using SPSS 20 software. The Kolmogorov-Smirnov test was used to determine the distribution of quantitative variables. The Mann-Whitney and Chi-square tests were used to compare groups and the Spearman coefficient to investigate the correlations between the variables. Data were expressed as median and interquartile range, and a value of $p < 0.05$ was considered significant.

Results

Table 1 shows the clinical and metabolic characteristics of the studied children. Obese children had significantly higher BMI, WC, insulin, HOMA, and triglyceride values than the control group. However, no differences were found in blood glucose, total cholesterol, HDL, and LDL cholesterol levels. Abdominal obesity was present in 100% of obese children.

The analysis of family history of the studied children showed that 71% (n = 26) had a history of obesity, diabetes and high blood pressure, being diabetes the most frequent disease, regardless presence/absence of concomitant obesity or high blood pressure (51%, n = 19).

Obese children had significantly higher values of TNF-α, IL-6, MCP-1, sCD40L, and us-CRP than their control pairs with normal weight (Table 2). No gender differences were observed in these analysis. Similarly, these molecules presented no differences between prepubertal and pubertal children.

Interestingly, we found that 67% (n=25) of obese children presented IR. When comparing obese children with and without IR, there was no difference in proinflammatory molecules concentration.

Both BMI and WC were positively correlated with the variables MCP-1, sCD40L, insulin and HOMA index (Table 3).

Table 3. Correlations between the variables studied

	IMC		CC	
	r	p	r	p
MCP-1	0.42	0.003	0.41	0.007
sCD40L	0.47	0.001	0.52	0.001
TNF-α	0.36	0.17	0.34	0.25
IL-6	0.28	0.07	0.24	0.15
hsPCR	0.23	0.10	0.27	0.08
Insulin	0.64	0.0001	0.68	0.0001
HOMA	0.55	0.0001	0.61	0.0001

The Spearman correlation coefficient was applied and considered significanta $p < 0.05$.

Discussion

The role of chronic low-grade inflammation as a link between obesity and its cardiovascular consequences has been convincingly demonstrated in recent years[26]. Research in obese children and adolescents has shown vascular abnormalities such as decreased peripheral vascular reactivity, increased carotid artery intima-media thickness, and elevated levels of adhesion molecules[27-29].

In this study, serum levels of proinflammatory molecules in obese children and adolescents were investigated . The results showed that obese infant-juvenile population had significantly higher values of TNF-α, IL-6, and us-CRP compared to the control group, coincidentally with previous reports in the same kind of patients[30,31]. Different mechanisms could explain these results, for example, TNF-α and IL-6 promote lipolysis and the release of free fatty acids, contributing to increased liver glucose production and IR. Both cytokines also cause adipocyte differentiation and promote inflammation, not only in adipose tissue but also in endothelial and hepatic cells, stimulating the synthesis of CRP[32].

Likewise , the high MCP-1 values found in this study are consistent with those reported by other researchers[20,33]. MCP-1 has been studied in relation to inflammatory diseases such as diabetes and obesity. Accordingly , visceral and subcutaneous adipose tissue in obese patients presented an over-expression of MCP-1 gene compared with thin control patients[34]. Furthermore, MCP-1 expression was higher in omental fat than in subcutaneous fat in patients with severe obesity[35].

In addition, we have found higher levels of sCD40L in obese children, compared to control patients. The literature reports high values of this molecule in adults with acute coronary syndrome, hypercholesterolemia, diabetes and obesity[36,37], as well as in children with hypercholesterolemia and type 1 diabetes[38,39]. However, research on this association in obese children is sparse and discordant. Byun et al found lower values of sCD40L in obese people compared to control patients, as opposed to our study[40]. Further study would be necessary to explain these differences. Noteworthily, our correlation analysis indicates that obesity is associated with subclinical inflammation and IR.

The current study has some limitations, mainly considering that the cross-sectional design only allows for association, but not causality. The sample size is relatively small, and some results did not reach the verge of statistical significance, such as the difference between gender and pubertal development. Therefore, studies in a larger population are needed for further research.

In conclusion, elevated serum levels of TNF-α, IL-6, MCP-1, sCD40L, and us-CRP suggest the presence of a pro-inflammatory state in the studied population. These data provide preliminary results for Argentinean obese children, which should be confirmed with further research studies. Early assessment of these molecules is important in order to implement appropriate therapeutic approaches to prevent cardiovascular risk factors during childhood.

Ethical responsibilities

Human Beings and animals protection: Disclosure the authors state that the procedures were followed according to the Declaration of Helsinki and the World Medical Association regarding human experimentation developed for the medical community.

Data confidentiality: The authors state that they have followed the protocols of their Center and Local regulations on the publication of patient data.

Rights to privacy and informed consent: The authors have obtained the informed consent of the patients and/or subjects referred to in the article. This document is in the possession of the correspondence author.

Financial Disclosure

Authors state that no economic support has been associated with the present study.

Conflicts of Interest

Authors declare no conflict of interest regarding the present study.

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