Evaluation of the Relationship between Insulin Resistance and 8-Iso Prostaglandin Levels in Obesity Children

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Abstract

Introduction: The rising rate of childhood obesity and the serious health problems it causes are gaining increasing attention in medical research and health policy.

Aim: This study aimed to evaluate the relationship between insulin resistance and the oxidative stress biomarker 8-iso-prostaglandin F₂α levels in obese children.

Materials and methods: Forty-four children in total (21 boys and 23 girls) aged between 6 and 15 years and diagnosed with obesity who attended the Pediatric Endocrinology Unit between December 2020 and June 21 were enrolled in our study. Forty children (20 boys and 20 girls) without systemic diseases were selected as controls. From the percentile curves determined for Turkish children, percentile values of obese children and control group were calculated based on sex and age. In addition, the insulin resistance values (HOMA-IR) in the homeostasis model were calculated. The relationship between the variables was examined with the Pearson and Spearman correlation tests. Children between the 5th and 85th percentile were defined as the control group, and those above the 95th percentile were defined as the obese group. Systolic and diastolic blood pressure, triglyceride, total cholesterol, LDL cholesterol, HDL cholesterol, fasting blood sugar (glucose), insulin, and 8-iso-PGF₂α concentrations were measured in all children included in the study.

Results: There were significant differences between the two groups in terms of age, body mass index, and systolic and diastolic blood pressures (p<0.05). Glucose, triglyceride, insulin, 8-iso-PGF₂α, and HOMA-IR levels were found to be statistically significantly higher in obese children than the levels in the control group (p<0.05). In addition, significant positive correlations were found between insulin levels and glucose, triglyceride and HOMA-IR values in obese patients (p<0.05). In obese children, 8-iso-PGF₂α concentrations were found to be statistically significantly higher than those in the control group (p<0.01). ROC analysis had a good diagnostic value for 8-iso-PGF₂α where the area under the curve was 1.0. A direct, positive, statistically significant correlation was found between insulin resistance and the 8-iso-PGF₂α values (r=0.420, p=0.037).

Conclusions: 8-iso-PGF₂α concentrations were found to be higher in obese children than in the control group. It was observed that increased insulin resistance raised 8-iso-PGF₂α levels. 8-iso-PGF₂α is thought to be particularly important for the diagnosis and treatment of these patients, with 99% sensitivity and specificity.

Keywords

8-iso-PGF₂α, childhood obesity, oxidative stress, pediatric
INTRODUCTION

Obesity is defined as an increase in energy intake over energy expenditure and an increase in fat tissue in the body. Excessive energy intake causes the accumulation of adipose tissue in the body. Today, obesity is considered among the diseases that carry the most important risk of mortality and morbidity, together with the complications it brings. The World Health Organization (WHO) has ranked obesity in first place among the most important diseases. It is known that obesity causes a number of diseases, such as cardiovascular diseases, insulin resistance, oxidative stress, and dyslipidemia. An increased body mass index (BMI) poses a serious risk to body health, and those with a BMI above 30 are considered obese. Obesity directly leads to insulin resistance, increasing insulin levels in the blood. The need for more insulin to produce a normal biological response is called insulin resistance. Insulin resistance has been demonstrated in almost all type 2 diabetes patients.

Obesity has become a major health concern for both children and adults as a result of factors such as improved economic conditions, increased consumption of ready-made foods in recent years, children spending more time at home due to technological advancements, shrinking playgrounds, and climate change. According to some reports, one-third of obese children grow up to be obese adults. Furthermore, different disorders are observed in these people at an adult age. One of the issues that the health community is most concerned about is the fact that childhood obesity will rise in the twenty-first century.

Obesity has become more prevalent in Western societies, according to studies conducted on children aged 6 to 19. While the prevalence of being overweight is 13.9% on average in the children of our country, it has been reported that the average prevalence of obesity is 4.8%. However, a study on children in Turkey found that obesity was increasing in both sexes. Childhood obesity can be said to be attributed to genetic and familial factors, psychological factors, a sedentary lifestyle, and poor eating habits. Fatigue, difficulty in breathing, and extremity pain are observed in very few of obese children. It is observed that they have a high appetite, they eat carbohydrate and fat-based diets, and they are reluctant to consume fruit and vegetables.

Reactive oxygen species (ROS) play a role in the pathophysiology of many diseases, including cardiovascular diseases, obesity, type 2 diabetes, and atherogenic mechanisms. It is stated that oxidative stress is associated with the formation of adipose tissue, which contributes to the development of obesity and metabolic syndrome. The level of antioxidant capacity decreases with obesity and the increase in adipose tissue. In obesity, mechanical work force and myocardial mechanism increase and oxygen consumption accelerates. Increasing oxygen consumption accelerates the formation of ROS. Isoprostanes are products formed by the nonenzymatic peroxidation of polyunsaturated fatty acids such as arachidonic acid induced by free radicals. Isoprostane and prostaglandins are called 8-iso prostaglandin F2α because they are isomers of prostaglandin F2α formed by cyclooxygenase. 8-iso-PGF2α has been measured as an indicator of lipid peroxidation in various body fluids such as urine, blood, bile, pericardial fluid, cerebrospinal fluid, and in tissues such as the brain and liver. An indicator of oxidative stress and lipid peroxidation, has been studied in cardiovascular diseases, lung diseases and neurodegenerative diseases.

AIM

The study aimed to evaluate the relationship between insulin resistance and the oxidative stress biomarker 8-iso-PGF2α levels in obese children in our country where parent-child association is high.

MATERIALS AND METHODS

The study included 44 children, 21 boys and 23 girls, between the ages of 6 and 15, with obesity-related diagnoses who attended the Faculty of Medicine Pediatric Endocrinology Department at Kahramanmaras Sutcu Imam University between December 2020 and June 2021. The control group consisted of 40 children (20 boys and 20 girls) who were free of any systemic diseases.

Collection and storage of samples

Venous blood samples taken from the children in the patient and control groups in the morning after 8-12 hours of fasting were placed in tubes that did not contain anticoagulant substances. Then, the blood samples were centrifuged at 4000 rpm for ten minutes and their serum was separated with a Hettich centrifuge device. Glucose, triglyceride, total cholesterol, HDL cholesterol, and insulin analyses were performed. Furthermore, a sufficient amount of serum samples were separated for 8-iso PGF2α analysis and stored in a deep freezer at ~80°C until analysis.

Anthropometric measurements

Systolic and diastolic blood pressure measurements were made with an Oncomed brand sphygmomanometer on the right arm in a sitting position after 10 minutes of rest. Using height and weight measurements, percentile values of the control group and obese children were calculated according to sex and age. Homeostasis model assessment (HOMA) was used to determine insulin resistance.

\[
\text{HOMA-IR} = \frac{\text{Fasting insulin (U/ml)} \times \text{Fasting glucose (mmol/L)}}{22.5}
\]
Biochemical measurements

For the analysis of serum 8-iso-PGF$_{2\alpha}$ level, 8-iso-PGF$_{2\alpha}$ commercial kit (Cayman, USA, catalog No. 514638.2) enzyme immunoassay method was performed by ELISA using a 50-μl sample. The measurement principle is based on competitive enzyme immunoassay. Reagents were pipetted in the indicated amounts according to the experimental procedure. After pipetting, the test plate was covered and incubated at 4°C for 18 hours. After 18 hours, the plate was washed 5 times with the prepared washing solution. 200 μl of Ellman’s reagent solution was pipetted into all wells. The test plate was covered and incubated for 120 minutes on an orbital shaker at room temperature. At the end of the period, the test plate was read at a wavelength of 405-420 nm. The results of 8-iso-PGF$_{2\alpha}$ were expressed as pg/ml. Moreover, glucose, triglyceride, total cholesterol, and HDL-cholesterol concentrations were measured by enzymatic colorimetric methods on a synchron LX 20 analyzer (Beckman Coulter, USA) using original Beckman kits. The LDL-cholesterol levels were calculated with the Friedewald formula as follows:

\[
\text{Total cholesterol (mg/dl) = HDL-cholesterol + VLDL ([Trig]/5) + LDL-cholesterol}
\]

Statistical analysis

SPSS 15.0 statistical package program was used in the statistical evaluation of the findings. Independent t-test was performed for total cholesterol and LDL cholesterol. the Mann-Whitney U test, one of the nonparametric tests, was used for parameters that did not show normal distribution (blood pressure, serum glucose, triglyceride, HDL-cholesterol, 8-iso-PGF$_{2\alpha}$, and HOMA-IR indices). Relationships between parameters were evaluated in pairs with the Spearman correlation analysis. A value of \(p<0.05\) was considered statistically significant.

RESULTS

Considering the descriptive values, when the obese child and control groups were compared, it was seen that there was no difference between the two groups in terms of sex distribution and age. It was found that the height and weight of the obese children were significantly higher than those in the control group (Table 1).

Table 1. Descriptive values of the obese and control groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Obese children</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>N=44</td>
<td>N=40</td>
</tr>
<tr>
<td></td>
<td>23 (G), 21 (B)</td>
<td>20 (G), 20 (B)</td>
</tr>
<tr>
<td>Age</td>
<td>10.5±4.5</td>
<td>10.7±3.48</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>141.2±12.5</td>
<td>135.1±15.3</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>68.6±18.4</td>
<td>35.7±12.2</td>
</tr>
</tbody>
</table>

Table 2. Routine values of obese and control groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Obesity</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOMAR</td>
<td>2.15±0.76</td>
<td>7.51±2.11</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>BMI</td>
<td>17.10±2.32</td>
<td>32.02±4.53</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>81.50±7.78</td>
<td>112.30±12.73</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>121.18±16.42</td>
<td>150.27±37.07</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Insulin (µU/dL)</td>
<td>26±8.17</td>
<td>15.98±7.14</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>73.58±9.25</td>
<td>116.32±14.92</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>46.55±7.95</td>
<td>35.09±7.96</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>93.48±10.84</td>
<td>118.55±20.23</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Table 3. Values of 8-iso prostaglandin belonging to the control and obese groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Obesity</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>8-iso prostaglandin</td>
<td>10.29±1.23</td>
<td>27.15±8.41</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Significant differences were observed between the two groups in terms of age, body mass index, and systolic and diastolic blood pressures \((p<0.05)\). The systolic and diastolic blood pressure of the obesity and control groups are presented in Table 7. The lipid profiles of obese children and control groups were compared and TG, TC, and LDL values were found to be significantly higher in obese children compared to the control group, while HDL values were found to be significantly lower than the respective values in the control group (Table 2).

Obese children and control groups were compared in terms of 8-iso-PGF$_{2\alpha}$ levels which were found to be significantly higher in obese children than in the control group (Table 3).

A moderate correlation was found between HOMA and 8-iso-PGF$_{2\alpha}$ levels in obese children (Fig. 1).
When obese children and control groups were compared in terms of BMI and 8-iso-PGF$_{2\alpha}$ levels, BMI and 8-iso-PGF$_{2\alpha}$ values of obese children (32.03±4.53) were found to be significantly higher ($p$>0.001) compared to those in the control group (17.10±2.32) (Fig. 2).

Relationships between descriptive values, routine, and biochemical parameters were also examined in all children, and accordingly, a significant positive correlation was found with children's TG, HOMAR, BMI, glucose, LDL, TC, and 8-iso-PGF$_{2\alpha}$ levels. HDL and insulin levels were found to be significantly negatively correlated. The Spearman correlation analysis between groups is shown in Table 4. Since $p$<0.05, it was determined that the correlation coefficient was significant. Table 5 shows that 8-iso-PGF$_{2\alpha}$ levels are highly positively correlated with the control group and obesity.

Our ROC analysis showed that the measurement of 8-iso-PGF$_{2\alpha}$ showed a high level of accuracy (AUC=0.966) and a sensitivity of 100%, making it appropriate to trust it (Fig. 3, Table 6).

**DISCUSSION**

Obesity is a chronic, progressive disease characterized by psychological issues that limit physical activity. The prevalence of obesity is increasing rapidly in both developing and...
Table 6. ROC curves value for the obesity and control dimensions of 8-iso-prostaglandin F$_{2\alpha}$

<table>
<thead>
<tr>
<th>Risk factor AUC 95%</th>
<th>p</th>
<th>Cut-off</th>
<th>Sensitivity (%)</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.966 (0.927-1.00)</td>
<td>0.020</td>
<td>8.63</td>
<td>100</td>
<td>90.0</td>
</tr>
</tbody>
</table>

Table 7. Mean values of systolic and diastolic blood pressure in obese and healthy children

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Obesity</th>
<th>Control</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure (Hg)</td>
<td>140.18±26.78</td>
<td>118.26±26.78</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Diastolic blood pressure (Hg)</td>
<td>88.16±14.78</td>
<td>74.32±10.48</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>
8-iso-PGF2α levels were found to be significantly higher in obese children than in the healthy control group. In our research, we found that the triglyceride, total cholesterol, and LDL levels were significantly higher in obese children than those values in the control group. In addition, HDL levels were observed at lower levels compared to the control group. Our research result parameters are similarly supported by the study of Boyd et al. In addition, correlations between parameters were made in children and it was shown that there is a positive relationship between the triglyceride, total cholesterol, and LDL levels.

A study found that plasma 8-iso-PGF2 levels, which are used as an indicator of oxidative stress, were significantly higher in obese children than in the healthy control group. It is seen that there is a direct correlation between plasma 8-iso-PGF2α level and visceral adipose tissue. In addition, there was no significant correlation between subcutaneous adipose tissue and plasma 8-iso-PGF2 levels in non-obese children. It is stated that 8-iso-PGF2α is a strong and independent determinant of visceral adipose accumulation. In a study including 58 patients, Kelly et al. found that the plasma 8-iso-PGF2α levels of obese children were higher than those in normal children. High levels of plasma 8-iso-PGF2α can be interpreted as the possibility of a higher incidence of type 2 diabetes and cardiovascular diseases in obese children in the future. When the plasma 8-iso-PGF2α values are examined, it is seen that results consistent with the studies in the literature are obtained. Many researchers have reported an increase in plasma 8-iso-PGF2α levels in patients with insulin resistance. In another study evaluating the relationship of oxidative stress with diabetes, obesity and atherosclerosis, 8-iso-PGF2α levels were found to be significantly higher in obese adults. In a different study investigating the relationship of oxidative stress with obesity and insulin resistance only in adult males, it was reported that 8-iso-PGF2α levels were higher in obese males than in normal males. In the literature, it is seen that the obesity 8-iso-PGF2α correlation is mostly evaluated in adults. This study investigated the correlation between obesity in children and 8-iso-PGF2α.

The blood pressure values obtained in the study show that there is a correlation between 8-iso-PGF2α levels and obesity. While the mean systolic blood pressure was 140.18±26.78 mmHg in the obesity group, the mean systolic blood pressure was 118.26±26.78 mmHg in the control group. While the diastolic blood pressure was 88.16±14.78 mmHg in the children with obesity, it was 74.32±10.48 mmHg in the control group. It is known that 8-iso-PGF2α, which is administered endogenously into the vein, increases blood pressure. Therefore, increased 8-iso-PGF2α level in obese children is thought to be effective in increasing blood pressure.

CONCLUSIONS

Oxidative stress forms the infrastructure of many diseases. Today, exposure to oxidative stress due to various factors has decreased to very young ages. It has been proven by various studies that oxidative stress can disrupt the normal working principles of the energy production mechanisms in the body and affect the general working principles of the cell. In this context, understanding the metabolic and biochemical background of oxidant mechanisms in the development of diseases, interpreting their measurable results and identifying biomarkers will contribute to early diagnosis and treatment. There are many biomarkers used as indicators in oxidative stress studies. In the present study, 8-iso-PGF2α levels were based on as a biomarker of oxidative stress, and obese children were our area of interest. Our results showed that 8-iso-PGF2α concentrations were higher in obese children than in the control group. We can say that elevated 8-iso-PGF2α levels are related to high blood pressure and visceral adiposity. In this respect, we can say that increased levels of 8-iso-PGF2α which we consider as a biomarker of oxidative stress, may be a factor in the formation of cardiovascular diseases in obese children. Furthermore, we can say that the diagnosis of the disease can be made with 99% sensitivity of 8-iso-PGF2α levels in obese children. As insulin resistance increased, there was an increase in the 8-iso-PGF2α levels suggesting that this biomarker is important in the diagnosis of obesity.

Compliance with the ethical standards

All human studies have been approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. All persons gave their informed consent prior to their inclusion in the study.

Conflict of Interest

The authors declare that they have no conflict of interest.

Funding

The authors have no funding to report.

REFERENCES

Insulin Resistance of Obesity Children


Оценка взаимосвязи между резистентностью к инсулину и уровнями 8-Iso простагландинов у детей с ожирением

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Резюме

Введение: Растущий уровень детского ожирения и вызываемые им серьёзные проблемы со здоровьем привлекают все большее внимание в медицинских исследованиях и политике здравоохранения.

Цель: Это исследование было направлено на оценку взаимосвязи между резистентностью к инсулину и уровнями биомаркера окислительного стресса 8-изо-простагландина F2α у детей с ожирением.

Материалы и методы: В исследование были включены 44 ребёнка (21 мальчик и 23 девочки) в возрасте от 6 до 15 лет с диагнозом ожирение, которые посещали отделение детской эндокринологии в период с декабря 2020 года по 21 июня. В качестве контроля были отобраны 40 детей (20 мальчиков и 20 девочек) без системных заболеваний. Из кривых процентилей, определённых для турецких детей, были рассчитаны процентильные значения детей с ожирением и контрольной группы в зависимости от пола и возраста. Кроме того, были рассчитаны значения инсулинорезистентности (HOMA-IR) в модели гомеостаза. Взаимосвязь между переменными исследовали с помощью корреляционных тестов Пирсона и Спирмена. Дети между 5-м и 85-м процентилем были определены как контрольная группа, а дети выше 95-го процентиля были определены как группа с ожирением. У всех детей, включенных в исследование, измеряли систолическое и диастолическое кровяное давление, триглицериды, общий холестерин, холестерин LDL, холестерин HDL, уровень сахара в крови натощак (глюкоза), инсулин и концентрации 8-iso-PGF2α.

Результаты: Между двумя группами наблюдались значительные различия по возрасту, индексу массы тела, систолическому и диастолическому артериальному давлению (p<0.05). Установлено, что уровни глюкозы, триглицеридов, инсулина, 8-iso-PGF2α и HOMA-IR статистически значимо выше у детей с ожирением, чем в контрольной группе (p<0.05). Кроме того, были обнаружены значимые положительные корреляции между уровнем инсулина и показателями глюкозы, триглицеридов и HOMA-IR у пациентов с ожирением (p<0.05). Установлено, что у детей с ожирением концентрация 8-iso-PGF2α статистически значимо выше, чем в контрольной группе (p<0.01). ROC-анализ имел хорошую диагностическую ценность для 8-iso-PGF2α, где площадь под кривой равнялась 1.0. Обнаружена прямая положительная статистически значимая корреляция между резистентностью к инсулину и концентрациями 8-iso-PGF2α (r=0.420, p=0.037).

Заключение: Установлено, что у детей с ожирением концентрация 8-iso-PGF2α выше, чем в контрольной группе. Было замечено, что повышенная резистентность к инсулину повышает уровень 8-iso-PGF2α, 8-iso-PGF2α считается особенно важным для диагностики и лечения этих пациентов с чувствительностью и специфичностью 99%.

Ключевые слова
8-iso-PGF2α, детское ожирение, оксидативный стресс, педиатрический