THE ASSESSMENT OF THE RELATION BETWEEN IL-6 174 G/C, IL-6 190 G/C, IL-6 572 G/C GENES POLYMORPHISMS AND NUTRITIONAL STATUS DISORDERS IN A CHILD POPULATION FROM ROMANIA

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Abstract

Background Nutritional disorders are plurifactorial diseases in which the genetic aspects have an important role near the lifestyle and dietary habits. The systemic inflammation and cytokines levels are much studied in the etiopathogeny of these diseases. We aimed to assess the relation between IL6-174G/C, IL6-190T/C and IL6-572G/C genes polymorphisms and nutritional status in a group of pediatric patients.

Methods The study included 385 patients: 173 malnourished, 102 obese and 110 controls in terms of genetic, biochemical and anthropometric tests.

Results For IL6-174G/C gene, in obese, predominated of CG genotype (p=0.001); CC genotype was a protective factor, in malnourished, GG and CG genotypes (p=0.0001). Regarding IL6-190T/C gene, genotype CC was more frequent in obese (p=0.0001), in malnourished, CT heterozygotes (p=0.003), and TT genotype having protective function. Analysing IL6-572G/C gene polymorphisms, CC genotype was more frequent in obese (p=0.0001), in malnourished, CT genotype (p=0.003), and TT genotype having protective function. BMI, MUAC, TST and albumine levels correlated with CC genotype of IL6-190 and IL6-572 genes in obese, while in malnourished, they correlated with GG and CC genotypes of IL6-174 and IL6-572 genes and with CT genotype of IL6-190 gene.

Conclusion: Child nutritional disorders were more frequent associated with IL6-174 G allele carriers. The highest risk of developing obesity was found in C-carriers of IL6-572 gene; CC genotype of IL6-174 may be a protective factor for obesity, while IL6-190 TT genotype could have protective function for malnutrition. Further research would establish the role of certain gene constellations in nutritional status disorders.

Keywords: child, gene polymorphism, nutritional disorders.

Background

Nutritional disorders are plurifactorial conditions; it is proved that genetic factors are considerable involved in malnutrition and obesity;1,3 associations of various gene polymorphisms with nutritional status, systemic inflammation and cytokines levels is increasingly studied; IL-6 gene is located on chromosome 7p21 being known about five promoters of this gene polymorphisms with role in nutritional status modulation.3,4,5 We aimed to assess the relation between IL6-174 G/C, IL6-190 T/C and IL6-572 G/C genes polymorphisms and nutritional status in a group of pediatric patients.

Methods

The study included 385 patients hospitalized in a pediatric department between June 2011 to December 2013. After complete anthropometric asessment, including weight/W (kg), height/H (cm), Middle upper arm circumference /MUAC (cm), and Tricipital skinfold thickness/TST (mm) measurement, Body mass index/BMI (kg/m²) calculation, we performed the conversion of all parameters in SD (z-scores) for age and sex (using Growth Analyser software, available on (https://www.growthanalyser.org/); then the patients were divided depending on the BMI z-score in three groups: 173 malnourished and 102 obese children, which were compared with 110 controls in terms of genetic aspects and biochemical tests. Obesity was defined as BMI-for-age above the 95th percentiles or over +2.0 SD (World Health Organization standards),4,7 while malnutrition was considered at BMI below −2.0 SD.5 Patients with specific chronic conditions that could influence nutritional status, with an acute infection, those whom complete (clinical, biochemical and genetic) assessment could not be performed or whose parents did not agree to participate in the study (did not sign the informed consent) were excluded from these research. The study was approved by the Ethics of Research Committee from the University of Medicine and Pharmacy Targu Mures.

From laboratory tests, proteins, albumins, total cholesterol and triglyceride levels were assessed, biochemical metabolic parameters being known as the best characterisation of the nutritional state. We also aimed to establish which of the adipokines [interleukins (IL6, IL8) leptin and adiponecint] is better correlated with nutrititional disorders and also with IL
genes polymorphisms. For genetic polymorphisms, DNA was obtained from whole blood samples (Quick-gDNA MiniPrep kit). The IL6-572G/C and 190T/C genotypes were determined using polymerase chain reaction (PCR) followed by restriction fragment length polymorphism assay (PCR-RFLP)\(^1\), while the IL6-174G/C genotypes were determined by amplification refractory mutation system- PCR (ARMS-PCR) method.\(^9\)

Specific statistical tests were applied (Graph Pad 3.6 State Software, San Diego, California, USA).

Results

**The anthropometric characteristics of the subjects in the study groups**

The control group included 110 healthy children with median BMI -0.03 SD and median age 10.90 years; in the malnourished group there were 173 patients with median BMI -2.17 SD and median age 10.7 years; the obese group comprised 102 obese children, with median BMI 2.61 SD, and median age 9.61 years. There was no statistically significant difference between the median ages of the three groups (p=0.09), so the study groups were thus comparable (Table 1).

Table 1. The descriptive analysis of anthropometric parameters in children with nutritional disorders compared to controls

<table>
<thead>
<tr>
<th>Variabile</th>
<th>Control group (n = 110)</th>
<th>Malnourished group (n = 173)</th>
<th>Malnourished vs controls p value</th>
<th>Obese (n = 102)</th>
<th>Obese vs controls p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (F/M), no</td>
<td>71/39</td>
<td>43/59</td>
<td>0.001</td>
<td>43/59</td>
<td>0.001</td>
</tr>
<tr>
<td>Age (median-range)</td>
<td>10.9 (1-18)</td>
<td>10.7 (1-18)</td>
<td>0.09</td>
<td>9.7 (1-17)</td>
<td>0.07</td>
</tr>
<tr>
<td>W SD (median-range)</td>
<td>-0.08 (-3.7-4.2)</td>
<td>-1.3 (-7.0-6.3)</td>
<td>0.0001</td>
<td>2.9 (-1.4-7.8)</td>
<td>0.0001</td>
</tr>
<tr>
<td>H SD (median-range)</td>
<td>-0.4 (-3.2-2.8)</td>
<td>-1.16 (-8.6-6.04)</td>
<td>0.005</td>
<td>0.7 (-2.4-4.7)</td>
<td>0.0001</td>
</tr>
<tr>
<td>BMI SD (median-range)</td>
<td>-0.03 (-1.84-1.9)</td>
<td>-2.17 (-8.9-2)</td>
<td>0.0001</td>
<td>2.61 (2.6-18)</td>
<td>0.0001</td>
</tr>
<tr>
<td>MUAC SD (median-range)</td>
<td>-0.25 (-5.3-7.5)</td>
<td>-2.2 (-9.1-5.3)</td>
<td>0.0001</td>
<td>4.5 (-1.3-13.2)</td>
<td>0.0001</td>
</tr>
<tr>
<td>TST SD (median-range)</td>
<td>-0.8 (-5.9-9.01)</td>
<td>-2.2 (-8.4-4.5)</td>
<td>0.0001</td>
<td>2.8 (-1.3-8.2)</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

We obtained statistically significant differences between the median BMI of the groups with nutritional disorders compared to controls (p=0.0001 both for malnourished and obese), which derive naturally from the batches structure. The same, the values of all anthropometric measurements (weight, height, MUAC, TST) were significantly higher in the obese group (p=0.0001), and significantly lower in malnourished (p=0.0001) in comparison with controls (data showed in Table 1).

**The biochemical characteristics of the subjects in the study groups**

Total protein and albumin levels were significantly lower in malnourished children (p=0.0001; p=0.0003), as well as total cholesterol and HDL-cholesterol levels (p=0.01; p=0.004), while there were higher values of GPT in the malnourished group (p=0.01). In the obese group, higher values of GPT were observed (p=0.01); on the other hand, no statistically significant differences were found for other biochemical tests between obese and control group, although total cholesterol, LDL, TG, GOT and protein levels were higher in obese, and HDL was decreased compared to the average amount detected in the control group.

Table 2. The descriptive analysis of biochemical parameters in children with nutritional disorders compared to controls

<table>
<thead>
<tr>
<th>Variabile</th>
<th>Control group (n = 110)</th>
<th>Malnourished group (n = 173)</th>
<th>Malnourished vs controls p value</th>
<th>Obese (n = 102)</th>
<th>Obese vs controls p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood sugar (media±SD)</td>
<td>83.3±10.8</td>
<td>82.7±13.1</td>
<td>0.55</td>
<td>87.7±17.7</td>
<td>0.05</td>
</tr>
<tr>
<td>Total protein (media±SD)</td>
<td>7.4±0.66</td>
<td>6.7±0.87</td>
<td>0.0001</td>
<td>7.5±0.48</td>
<td>0.14</td>
</tr>
<tr>
<td>Albumin (media±SD)</td>
<td>4.36±0.53</td>
<td>4.08±0.67</td>
<td>0.0003</td>
<td>4.66±0.47</td>
<td>0.0001</td>
</tr>
<tr>
<td>T Chol (media±SD)</td>
<td>158.1±28.3</td>
<td>149.9±31.2</td>
<td>0.01</td>
<td>165.7±38.1</td>
<td>0.17</td>
</tr>
<tr>
<td>HDL (media±SD)</td>
<td>53.0±13.5</td>
<td>47.2±18.3</td>
<td>0.004</td>
<td>52.3±14.7</td>
<td>0.66</td>
</tr>
<tr>
<td>LDL (median-range)</td>
<td>93 (49-216)</td>
<td>85 (32-151)</td>
<td>0.07</td>
<td>91 (42-216)</td>
<td>0.52</td>
</tr>
<tr>
<td>TG (median-range)</td>
<td>67.5 (26-268.7)</td>
<td>63.5 (31.3-270.9)</td>
<td>0.52</td>
<td>83.9 (34.1-450)</td>
<td>0.06</td>
</tr>
<tr>
<td>GOT (median-range)</td>
<td>26 (12.4-87.3)</td>
<td>30.9 (10.4-106.0)</td>
<td>0.01</td>
<td>24.6 (13-178)</td>
<td>0.65</td>
</tr>
<tr>
<td>GPT (median-range)</td>
<td>17 (7-77)</td>
<td>21.6 (10-342)</td>
<td>0.78</td>
<td>21.6 (10-342)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

T Chol – total cholesterol; HDL - -; LDL - -; TG - -; GOT - -; GPT - -

The descriptive analysis of genetic parameters of the subjects in the study groups

For IL6-174 G/C gene, in malnourished, GG and CG genotypes were more frequent (p=0.0001); in obese, there was a predomination of CG genotype (p=0.001), while CC genotype was a protective factor (Table 3).
Regarding IL6-190 T/C gene, CT heterozygotes were more frequent in malnourished (p=0.003), TT genotype had protective function against malnutrition; genotype CC was more frequent in obese (p=0.0001) (Table 3).

Analysing IL6-572 G/C gene polymorphisms, GG genotype predominated in both malnourished and controls; CC and CG genotypes were more frequent in obese (p=0.005 and 0.0001, respectively) (Table 3).

If we take under consideration the allele distribution, for polymorphism of IL6-174 gene, C carriers were more frequent in control group than in malnourished, with statistical significance, but because the percentage was lower in malnourished children, C carriers are not the ones associated with malnutrition, but the GG genotype (which has the highest percentage in this nutritional disorder, p=0.0001, OR=0.25).

In obese, C carriers were present in 88.2% of cases, but without statistically significance (p=0.08) (Table 4). For IL6-190 gene, C carriers (CC+CT) was also more frequent in malnourished group, with a significant p of 0.0001, OR=7.7. In obese, C carriers were also increased in frequency, statistically significant (p=0.04, OR=2.3) (Table 4).

For IL6-572 gene, C carriers (CC+CG) genotypes were associated with malnutrition, but statistically insignificant (p=0.26, OR=5.8), and C carriers were statistically significant associated with obesity (p=0.0001; OR=5.8) (Table 4).

The relations between anthropometric, biochemical and genetic parameters

BMI, MUAC, TST and albumin levels correlated with CC genotype of IL6-190 and 572 genes in obese; in malnourished, they correlated with GG and CC genotypes of IL6-174 and IL6-572 genes and with CT genotype of IL6-190 gene.

The albumin levels were significantly lower in the malnutrition group for the CC, CT and TT genotypes of IL6-190 gene (p=0.0001), as well as for GG and CC genotypes of IL6-572 gene (p=0.0001); significantly lower protein level in malnutrition group were found in CC and CT genotypes of IL6-190 gene.

The leptin level (an important marker of inflammation) was significantly higher in the obese group for all three genotypes of IL6-174, IL6-190 and IL6-572 genes (with statistically significantly higher risk for CG genotype of IL6-174 gene, and for all three genotypes of IL6-190 and IL6-572 genes, p=0.0001).

The adiponectin level was negatively associated with BMI, being higher in the control group and lower in the obese group, with statistical significance for the CG genotype of IL6-174 gene (p=0.002), for CT genotype of IL6-190 gene (p=0.0009), and for the CG genotype of IL6-572 gene (p=0.0001).

Discussions

The genetic aspects from our research have not been previously studied in children with nutritional disorders; there are a few studies which assessed the role of genes potentially involved in obesity – especially in adults, and only a few isolated study who watched genetic aspects of interleukin genes associated with malnutrition - and those also just in adult populations.14 1013 The main finding of our work is that the polymorphisms of the IL6-174 G/C, IL6-190 C/T, and IL6-572 C/G are associated with the risk of developing nutritional disorders in children.
We found significantly statistic associations for GG and CG genotype of IL6-174 polymorphism with malnutrition, similarly to data from other studies.\textsuperscript{1,12} Regarding the IL6-190 gene polymorphisms, we obtained a poor but significant association between malnutrition and CT genotype, and also a significant relation for TT genotype, but with a very low percentage in malnourished, comparatively with controls which means that the TT genotype is a protective factor against malnutrition, data similarly to other reports.\textsuperscript{1}

For IL6-572, we found statistical significance (p=0.0001) in the malnutrition group in comparison with the control group for MUAC, TST and serum albumins with a certain predominance of GG genotype.

The genotype distribution of the frequency of C allele polymorphism in obese children in comparison with normal-BMI children, were similar to data published in other studies.\textsuperscript{5,14} For the IL6-174 polymorphism, we found associations of obesity with CG genotypes, CC genotype being a protective factor for obesity; CC genotype was more frequent in obese for IL6-190 and IL6-572 genes (p=0.0001 in both cases).

**Conclusions**

Child nutritional disorders (malnutrition and obesity, respectively) were more frequent associated with IL6-174 C-allele carriers. The highest risk of developing malnutrition was found in children G carriers of IL6-190 gene, followed by the C carriers Il6-174 genotype polymorphism. IL6-190 TT genotype could have a protective function against malnutrition.

The highest risk of developing obesity was found in C-carriers of IL6-572 gene, followed by the C allele carriers of the IL6-190 gene polymorphism; CC genotype of IL6-174 may be a protective factor for obesity. Further research would establish the role of certain gene constellations in nutritional status disorders.

**References**


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