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Session 4: Dietary strategies to prevent and mitigate inflammatory diseases

Inflammatory biomarker profile in children with cystic fibrosis: preliminary study

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The aim of this preliminary study was to determine specific proteins, related to inflammation process and nutritional status as well as to total antioxidant capacity, in children suffering from cystic fibrosis (CF). The study was performed on 17 nonhospitalized children (12 boys and 5 girls) with CF aged 3 months to 10 years, who were assisted at the Nutrition Service from Pedro de Elizalde Hospital. Transferrin, transthyretin, ceruloplasmin (Cp), haptoglobin, C-reactive protein (CRP) and fibrinogen were measured by single radial immunodiffusion techniques. Total antioxidant capacity (TAC) was determined by a decolorization assay. Statistical analyses were performed by the Student’s t test. Transferrin and transthyretin values were lower in CF patients in comparison with data obtained from healthy children (reference group, RG). The decreased transferrin concentration and the tendency towards low plasma transthyretin values suggested an abnormal nutritional status. However, higher Cp and haptoglobin levels were shown in patients than in RG. The fact that 23 and 50% of patients exceeded the desirable values for fibrinogen (<285.0 mg/dl) and CRP (<0.2 mg/dl), respectively, should be highlighted. The TAC (nm; Trolox equivalents) was shown to be lower in the CF group than in RG. The diminished TAC concomitant with an increased plasma Cp concentration would exacerbate the inflammatory status and could explain the depression of the immune system. These preliminary results could explain the need to include biochemical and functional parameters in the early nutritional status evaluation in CF patients in order to use appropriate nutritional and pharmacological therapies and consequently to improve their survival and quality of life.

Cystic fibrosis: Children: Biochemical parameters: Proteins: Total antioxidant capacity

Cystic fibrosis (CF) is an inherited disease characterized by an abnormality in the body’s salt, water and mucus-making cells. As the movement of salt and water in and out of cells is altered, mucus becomes thickened. This fact can affect many organs and body systems including the following: the respiratory system – sinuses and lungs; the digestive system – pancreas, liver, gallbladder and intestines; the reproductive system – especially in males, where sperm-carrying ducts become clogged; and sweat glands(1).

In children with CF, this mucus can also prevent the normal absorption of essential nutrients and fat in the gut, leading to poor digestion, slow growth, impairment of weight gain, greasy bowel movements and increased vulnerability to infections. Diminished secretion of pancreatic enzymes is the main cause of poor growth, fatty diarrhoea and deficiency in fat-soluble vitamins(2,3).

Nutrition plays an essential role in the management of CF, particularly in infants with their high energy

Abbreviations: CF, cystic fibrosis; Cp, ceruloplasmin; TAC, total antioxidant capacity; TTR, transthyretin.

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Material and methods

The study was performed on 17 non-hospitalized children (12 boys and 5 girls) with CF aged 3 months to 10 years, who were assisted at the Nutrition Service from Pedro de Elizalde Hospital. Blood samples were collected from fasting patients. Transferrin, transthyretin (TTR), ceruloplasmin (Cp), haptoglobin, C-reactive protein and fibrinogen were measured by single radial immunodiffusion techniques by using commercially available kits (Diagnostica Stago, France) (11). TAC was determined by a decolorization assay applicable to both lipophilic and hydrophilic antioxidants, including flavonoids, hydroxycinnamates, carotenoids and plasma antioxidants(12,13). Statistical analyses were performed using the Student’s t test.

The report was approved by the Ethics Committee from the University of Buenos Aires, and informed consent was obtained from parents before recruiting the patients into the study.

Plasma fractions results were expressed as X with standard deviation and were compared using the Student’s t test with published data obtained in our laboratory from healthy children of both sexes and a similar age range (reference group)(14,15). C-reactive protein and fibrinogen concentrations were compared with the desirable values provided by international bibliography. TAC reference value was obtained in healthy children assessed in the same hospital for periodic control (M. Insani, unpublished results).

Results

When the population tested was divided by age range (3 months–2 years and >2 years), no differences were observed in all the studied biomarkers. That is the reason why we analyzed the population as a whole.

Lower values of the nutritional status-related plasma proteins (transferrin and TTR) were found in CF patients (Table 1) in comparison with data obtained in our laboratory from healthy children of both sexes and a similar age range (reference group) (P<0.01), 50% of children showing TTR values below 20-0 mg/dl, which is related to protein deficiency(16). However, both Cp and haptoglobin levels were found higher in CF patients in relation to the reference group. It should be highlighted that 23 and 50% of patients exceeded the desirable values for fibrinogen (<285-0 mg/dl) and C-reactive protein (<0.2 mg/dl), respectively.

TAC(12,13) was significantly lower in CF patients than in healthy children.

Discussion

Previous findings by our working group in children suffering from CF suggest that the immune system, evaluated through the levels of C3c and C4c fractions and the activity of adenosine deaminase (a T lymphocyte-related enzyme) was altered(17). Moreover, the decreased transferrin concentration and the tendency towards low plasma TTR values found in this preliminary report suggested an abnormal nutritional status(16). TTR shows the same behaviour reported in adults suffering from this pathology, but adult patients show higher transferrin levels than children(18). In acute phase reactions such as infections and chronic inflammation (situations observed in children suffering from CF), transferrin concentration could be reduced. Moreover, TTR synthesis is inhibited by TNFα; this outcome could explain the concentration levels of this protein fraction found in children suffering from CF(19).

In addition, the observed increase in Cp and haptoglobin plasma levels could be related to a concomitant inflammatory status; in fact, the increased synthesis of these acute phase proteins has been shown to be induced by pro-inflammatory cytokines, such as IL-6, IL-1 and TNFα(19). The CF group showed higher levels of fibrinogen and C-reactive protein together with lower values of HDL-cholesterol (27-8 (so 10-3) mg/dl) than in the reference group; this result would confirm an inflammatory status in children at risk of CVD(20); moreover, the described

Table 1. Studied biochemical parameters and total antioxidant capacity (TAC) (mean values and standard deviation)

<table>
<thead>
<tr>
<th>Plasma levels (mg/dl)</th>
<th>CF</th>
<th>RG</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean</strong></td>
<td><strong>SD</strong></td>
<td><strong>Mean</strong></td>
</tr>
<tr>
<td>Transferrin (Tf)</td>
<td>198±1*</td>
<td>53.2</td>
</tr>
<tr>
<td>TTR</td>
<td>18±8</td>
<td>6.1</td>
</tr>
<tr>
<td>Cp</td>
<td>49±2*</td>
<td>18.5</td>
</tr>
<tr>
<td>Haptoglobin</td>
<td>121±9*</td>
<td>87±0</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>266±3</td>
<td>145±7</td>
</tr>
<tr>
<td>CRP</td>
<td>2±2</td>
<td>1.0</td>
</tr>
<tr>
<td>HDL</td>
<td>198±1</td>
<td>53.2</td>
</tr>
<tr>
<td>TAC (mV; Trolox equivalents)</td>
<td>1±91*</td>
<td>0.04</td>
</tr>
</tbody>
</table>

CF, cystic fibrosis group; RG, reference group; TTR, transthyretin; Cp, ceruloplasmin; CRP, C-reactive protein.

*Significantly different at a level of P<0.01.

Further links exist between progression of CF lung disease and oxidative stress(9,10). Therefore, this preliminary study was aimed to determine specific plasma proteins related to inflammation and nutritional status, and total antioxidant capacity (TAC), in non-hospitalized children suffering from CF.
pro-oxidant activity of Cp could be considered as another independent risk factor for CVD in this population\(^{(21)}\). On the other hand, several studies have reported that inflammation is an event occurring prior to infection in patients with CF\(^{(22)}\).

Oxidative stress may play a significant role in the pathophysiology of CF. The lung, the main organ responsible for morbidity and mortality in this disease, is particularly vulnerable to high levels of oxidative stress\(^{(10,23)}\). The diminished TAC in non-hospitalized children concomitant with an increased plasma Cp concentration would exacerbate the inflammatory status and could explain the depression of the immune system due to the deterioration of the immune cells and/or their function.

**Conclusions**

Careful nutritional management has an important effect on growth and survival rates in CF (a complex disease that requires clinical care); the abnormalities in the oxidant–antioxidant balance raise several possibilities for therapeutic interventions concomitant with specific nutritional support adjusted to individual needs. Long-term studies are required to examine the effects of nutritional interventions on key clinical outcomes in CF\(^{(23)}\).

These preliminary results performed on low specific half-life plasma fractions related to both inflammation and nutritional status (transferrin 7–10 days, TTR 1–2 days, haptoglobin 2–4 days) could explain the need to include these biochemical and functional parameters in the early evaluation of non-hospitalized CF patients. This inclusion would allow us to analyse the patient’s nutritional and inflammatory evolution and to design appropriate nutritional and pharmacological therapies, these actions being able to improve the survival and life quality of these patients.

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