Plasma Insulin-like Growth Factor-1, Type 1 Procollagen, and Serum Tumor Necrosis Factor α in Children Recovering From Trichuris Dysentery Syndrome

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ABSTRACT. Objectives. To explore: 1) the relationship between plasma insulin-like growth factor-1 (IGF-1) and other markers of growth; and 2) the effect of serum concentrations of tumor necrosis factor α (TNF) on growth variables in children (2–10 years) stunted by Trichuris dysentery syndrome (TDS), recovering cases, and their matched controls.

Method. Fourteen patients with TDS were admitted to the Tropical Metabolism Research Unit, treated with albendazole and iron, and then followed with matched controls (n=28) for 1 year. Anthropometric and biochemical measurements were done on admission and then every 3 months for the year. Plasma IGF-1, the carboxyterminal propeptide of type 1 procollagen, serum TNF, total serum protein, serum albumin, and complete blood count were determined.

Results. Low admission plasma levels of IGF-1 in TDS cases were accompanied by high serum levels of TNF, and total serum protein, normal serum albumin, low hemoglobin, reduced collagen synthesis (low plasma carboxyterminal propeptide of type 1 procollagen), and growth failure. These variables improved significantly after treatment. Plasma levels of IGF-1 were significantly related to the Z-scores for height-for-age (r=0.60, 0.73, 0.68) and weight-for-age (r=0.69, 0.80, 0.69) of cases and controls, height-for-age (r=0.51, 0.52, 0.54) and weight-for-age (r=0.51, 0.52, 0.54) at each measurement throughout the year. Serum levels of TNF were not related to any of the growth variables.

Conclusion. These findings may contribute to the understanding of growth failure in children affected by other forms of chronic inflammatory bowel disease. Pediatrics 1999;103(5). URL: http://www.pediatrics.org/cgi/content/full/103/5/e69; Trichuris dysentery syndrome, insulin-like growth factor-1, type 1 procollagen, tumor necrosis factor α.

ABBREVIATIONS. TDS, Trichuris dysentery syndrome; IGF-1, insulin-like growth factor-1; PICP, the carboxyterminal propeptide of type 1 procollagen; TNF, tumor necrosis factor α; TSP, total serum protein; SA, serum albumin; CBC, complete blood count; HC, head circumference; MUAC, mid-upper arm circumference; CV, coefficient of variation; RIA, radioimmunoassay; CI, confidence interval.

Chronic infection with the geohelminth Trichuris trichiura in children is significantly associated with anemia and poor physical and cognitive development. Recent studies in Jamaica have shown a prevalence rate of 38% in rural schools. Trichuris dysentery syndrome (TDS) is the most severe form of Trichuris infection. Severe growth impairment occurs in the affected children. The children have chronic diarrhea with blood and mucus, iron deficiency anemia, anorexia, geophagia, and lower abdominal pain. Clubbing of fingers and toes may also be present. Sometimes there is a prolapsed rectum with worms adhering to the mucosa. Growth failure is reversed after the successful treatment of the helminthiasis although the diet remains unchanged. Cooper et al suggested that the pattern of catch-up growth in TDS points to the existence of some specific link between inflammation or allergy in the lower intestinal tract and the suppression of linear growth.

Insulin-like growth factor-1 (IGF-1) is considered a good biochemical marker for the evaluation of normal growth, growth disorders, and nutrition in children. It is a growth hormone-dependent peripheral effector of the anabolic actions of growth hormone especially skeletal growth and hematopoiesis. IGF-1 is a 70-amino acid mitogenic polypeptide structurally homologous to proinsulin. It is synthesized in the liver and also in cells of mesenchymal origin such as fibroblasts, smooth muscle cells, and glomerular mesangial cells. Plasma IGF-1 concentration increases during childhood, reaching a peak during puberty. There is a wide range of reference values for each gender and age group. The response to IGF-1 in tissues may be modulated by high-affinity IGF-1 binding proteins, six of which have been identified.

Type 1 collagen accounts for more than 90% of the organic matrix of bone. Circulating levels of the type 1 procollagen carboxyterminal propeptide (PICP) reflect the synthesis of type 1 collagen. A relationship has been shown between serum levels of PICP and height velocity. Tumor necrosis factor α (TNF)/cachectin, a macrophage-induced pleiotropic cytokine, has been implicated in the metabolic derangements of undernutrition and growth failure. Increased serum concentrations of TNF have been reported in children affected by TDS.

There is a systemic as well as a mucosal compo-
ment of the response to infection with *T trichiura*. TDS is associated with an acute phase plasma protein response. Cooper et al.\textsuperscript{13} reported a high median plasma viscosity during the first 6 months after treatment in 53 children affected by TDS. In these children, 39\% of the variance in plasma proteins was explained by plasma levels of fibrinogen and total globulin. A low mean SA was reported by Gilman et al.\textsuperscript{15}

In the complete blood count (CBC) there is a microcytic iron deficiency anemia and a nonspecific leukocytosis in TDS.\textsuperscript{3}

The objectives of this study were to explore 1) the relationship between plasma IGF-1 and other markers of growth; and 2) the effect of serum concentrations of TNF on growth variables in children (2–10 years) stunted by TDS, recovering cases, and their unaffected matched controls.

**METHODS**

**Study Participants**

Fourteen patients with TDS (2–10 years; mean age, 4.19 years; 8 boys and 6 girls), were diagnosed by colonoscopy, admitted to, and treated at the Tropical Metabolism Research Unit, University of the West Indies. The *Trichuris* was expelled in the hospital after treatment with albendazole (400 mg/day for 3 days) and each child was given a course of iron (60 mg FeSO\textsubscript{4} twice daily for 21 days). The children were then followed as outpatients along with matched controls (n = 28) for 1 year. The controls were matched for age (±6 months), gender, socioeconomic status, and neighborhood.

Anthropometric measurements, height, weight, head circumference (HC), mid-upper arm circumference (MUAC), and triceps and subscapular skinfold thicknesses were conducted on each child on admission and then every 12 weeks for 1 year. Additional measurements were done on cases at 3, 6, and 9 weeks after discharge. A single trained measurer (E.M.W.D.) used the same methods and equipment (validated on each measuring day) throughout the study.\textsuperscript{14} The mean percent coefficient of variation (%CV) of the measurements were: height = 3.8\%, HC = 1.7\%, MUAC = 2.3\%, triceps skinfold thickness = 6.7\%, and subscapular skinfold thickness = 3.9\%.

On admission and at each visit blood was drawn by venipuncture for CBC, plasma determinations of IGF-1, and PICP. (EMLA topical anesthetic cream [Astra Pharmaceuticals, UK] was applied to the venipuncture site before sampling to avoid discomfort to the child.)

Serum determinations of TNF by radioimmunoassay (RIA), total serum protein (TSP) and albumin (SA) by Abbott VP (Chicago, IL) analyzer, were done on the TDS cases, one sample on admission and two during recovery. One serum sample from each of the control children was used to determine reference values. A TDS child was considered to be recovering when his or her height velocity reached the >90th centile, +1.8 standard deviation.\textsuperscript{17}

The study was approved by the ethics committee of the Faculty of Medical Sciences, University of the West Indies, and the Ministry of Health, Jamaica. Before the start of the study, an informed written consent was signed by a parent and/or guardian of each child. Children with a history of asthma, sickle cell disease, heart disease, current chronic infections, severe illness, or known growth disorders were excluded from the study.

**Laboratory Procedures**

The plasma and serum samples were stored at −20°C after the addition of a protease inhibitor, aprotinin 5 μL/mL. All samples were assayed in duplicate.

**IGF-1**

IGF-1 was extracted from 250 μL of plasma using an octadecylsilica silica column extraction procedure. Levels of IGF-1 were determined by RIA using an Incstar IGF-1 \textsuperscript{125}I RIA Kit (Stillwater, MN). The interassay variation for normal values was as follows: low values, %CV = 8.6; high values, %CV = 10.7.

**PICP**

PICP was determined from 50 μL of plasma using a PICP \textsuperscript{125}I RIA equilibrium RIA kit from Incstar (Stillwater, MN). The interassay variation for normal values was as follows: low values, %CV = 2.8; high values, %CV = 13.0.

**TNF**

A BIOTRAK TNF α \textsuperscript{125}I RIA system from Amersham (UK) (Little Chafont, Buckinghamshire, UK) was used to measure the serum levels of TNF on 100 μL of serum.

Data were reduced using a Microsoft RIA curve-fitting program. Assays were repeated on any samples showing an intrasay CV >10%.

**TSP and Serum Albumin (SA)**

TSP and SA were determined using an Abbott VP (Chicago, IL) analyzer. CBCs were done by the Department of Hematology, the University of the West Indies.

**Data Analysis**

Height-for-age, weight-for-age, and weight-for-height Z-scores were calculated using the Centers for Disease Control and Prevention (Atlanta, GA) Anthropometric Software Package 1987 (CASP). Multivariate analysis of variance trend analysis was used to determine any significant trends in the serially measured variables. Paired *t* tests and Wilcoxon matched pairs signed nonpara-

| TABLE 1. Comparison of Height-for-Age, Weight-for-Age, and Weight-for-Height (Z-scores NCHS) of *Trichuris* Dysentery Syndrome Cases and Controls, on Admission, 6 months, and 1 year* |
|-------------------|--------------|---------------|--------------|-------------------|--------------|---------------|--------------|
|                   | Z-score      | Standard      | Minimum      | Maximum          | Z-score      | Standard      | Minimum      | Maximum          |
| Height-for-age    |              | Deviation     |              |                  |              | Deviation     |              |                  |
| Admission         | −2.79†       | 1.54          | −5.08        | 0.65             | −0.40        | 1.01          | −2.22        | 2.73             |
| 6 mo              | −2.37†       | 1.11          | −4.36        | 0.97             | −0.27        | 1.11          | −2.41        | 3.00             |
| 1 y               | −2.16†       | 1.26          | −4.33        | 0.38             | −0.40        | 1.45          | −1.93        | 5.25             |
| Weight-for-age    |              |               |              |                  |              |               |              |                  |
| Admission         | −2.75†       | 1.11          | −4.22        | 0.16             | −0.60        | 0.97          | −1.86        | 2.23             |
| 6 mo              | −1.94†       | 0.91          | −3.10        | 0.39             | −0.47        | 1.01          | −1.68        | 2.81             |
| 1 y               | −1.86†       | 0.83          | −3.17        | −0.38            | −0.34        | 1.22          | −1.93        | 4.04             |
| Weight-for-height |              |               |              |                  |              |               |              |                  |
| Admission         | −1.53†       | 0.93          | −3.37        | −0.02            | −0.37        | 0.78          | −1.57        | 1.33             |
| 6 mo              | −0.75        | 0.66          | −1.69        | 0.28             | −0.32        | 0.71          | −1.40        | 1.47             |
| 1 y               | −0.70        | 0.72          | −2.13        | 0.39             | −0.28        | 0.73          | −1.56        | 1.21             |

* Independent samples *t*-tests.
† Significant differences between cases and controls, *P* < .001.
TABLE 2. Comparison of Plasma Levels of IGF-1 (nmol/L) Between TDS Cases and Controls

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Cases (n = 14)</th>
<th>Controls (n = 28)</th>
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<th>P</th>
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<tr>
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<tr>
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<tr>
<td>Range</td>
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<tr>
<td>6 mo</td>
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</tr>
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<td>14.70</td>
<td>-2.38</td>
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<td>10.80, 21.34</td>
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<td>15.27</td>
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<td>11.26, 18.37</td>
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<tr>
<td>Range</td>
<td>4.02, 23.58</td>
<td>5.20, 26.52</td>
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</tbody>
</table>

Abbreviations: IGF-1, insulin-like growth factor-1; TDS, Trichuris dysentery syndrome; LQ, lower quartile; UQ, upper quartile.
* Mann-Whitney U test.
† Significant differences between cases and controls.

metric tests were used to compare the within-group variables. Independent samples, t tests, and Mann-Whitney U nonparametric tests were used to compare between-group variables. Spearman’s correlation coefficient and simple linear regression analysis were used to determine any relationships among the variables. The Statistical Package for the Social Sciences (SPSS, Chicago, IL) Version 6.0 was used.

RESULTS

Anthropometric Markers of Growth

On admission, the mean Z-scores (National Child Health Statistics) of the TDS children were significantly lower than the controls. Mean height-for-age Z-score = -2.79, 95% confidence interval (CI) -5.79, 0.21, P < .001; mean weight-for-age Z = -2.75, 95% CI -4.95, -0.55, P < .001; and mean weight-for-age Z = -1.53, 95% CI -3.33, 0.27, P < .001. The mean Z-scores of the controls were -0.40, -0.60, and -0.37, respectively (Table 1).

The mean MUAC of the cases was 14.41 cm (95% CI 11.81, 17.01), significantly lower than the controls, 16.53 cm (95% CI 14.29, 18.77; P < .001), until 9 months after treatment. There were no significant differences in mean skinfold thicknesses. The mean HC of the cases, 47.42 cm (95% CI 43.42, 51.42), was also significantly lower than the controls, 50.15 cm (95% CI 46.65, 53.65; P < .001).

At the end of 1 year, the observed mean velocities in height, 8.82 cm/year (95% CI 4.42, 13.22; P = .004) and HC, 1.15 cm/year (95% CI -0.43, 2.73; P = .008) of the cases were significantly higher than the controls. Mean HC, 48.87 cm (95% CI 44.99, 52.75; P = .006), and mean Z-scores of the cases, height-for-age, -2.16 (95% CI -4.76, 0.44; P < .001), and weight-for-age, -1.86 (95% CI -3.46, -0.26; P < .001), were still significantly lower than the controls. There was however no significant difference in mean weight-for-height Z-scores between the cases (-0.70; 95% CI -2.1, 0.7) and the controls (-0.28; 95% CI -1.74, 1.18).

IGF-1

On admission, 57% of the cases were below the IGF-1 reference range for their age and gender. The plasma levels of the controls were within the reference range.18 The median admission plasma level of IGF-1 in the cases, 6.0 nmol/L (range, 1.74, 11.64) was significantly lower than that of the controls, 16.34 nmol/L (range, 4.72, 39.58; P < .001).

Four weeks after worm expulsion, the cases had a significant rise in their median plasma level of IGF-1 to 9.66 nmol/L (P = .002). The median plasma levels of IGF-1 remained below those of the controls throughout the year. The differences were significant at the 6- and 9-month measurements (Table 2). The range of values, although wide, were within the ranges for IGF-1 in boys and girls of 2 to 10 years.18 The high upper limits of the distribution were explained by outliers (Fig 1).
Associations With Plasma Levels of IGF-1

In both cases and controls, height-for-age Z-scores were associated with plasma levels of IGF-1 at the end of each interval. Weight-for-age Z-scores were similarly associated with plasma levels of IGF-1 (Fig 2A and B, Table 3).

IGF binding proteins were not measured so that any modulating effects could not be assessed.

PICP

On admission, the median plasma level of PICP in the cases, 124.45 ng/mL, was significantly lower than that of the controls, 131.4 ng/mL (P < .001). Three months after treatment, there was no significant difference between the cases and the controls. At 1 year, however, the median plasma level of PICP in the cases was higher than that of the controls and this approached significance (Table 4). There were no relationships found between plasma levels of PICP, the indices of growth, height, and weight velocities, nor any of the changes in them.

SA

Mean SA in the TDS cases on admission, 43.88 g/L (95% CI 37.38, 50.38); mean of the recovering cases, 46.22 g/L (95% CI 41.96, 50.48); mean of the controls, 45.72 g/L (95% CI 42.98, 48.46). These values were within the normal range.

CBC

On admission, all the cases had microcytic and hypochromic erythrocytes. Their mean admission hemoglobin concentration, 8.1 g/dL (95% CI 2.56,
Comparison of the Plasma Levels of PICP (ng/mL) at Admission, 6 Months, and 1 Year* Spearman’s correlation.

Abbreviation: IGF-1, insulin-like growth factor-1.

Mann-Whitney U test.

Trichuris mean weight-for-height Six weeks after treatment, the cases had a normal height velocity, which is characteristic of catch-up improvement in plasma levels of IGF-1 and PICP. Although the error in measuring skinfold thicknesses between cases and controls throughout. On admission and during reinfection, the cases showed a nonspecific leukocytosis. This slow recovery in muscle protein and hemoglobin synthesis may be partly explained by several factors: the comparatively low levels of IGF-1; iron deficiency anemia and reduced muscle mass as features of the cachectic state induced by TNF; and increased iron requirements during periods of rapid growth. Plasma levels of IGF-1 were significantly below that of the controls in height-for-age and weight-for-age Z-scores and HC. The mean MUAC of the cases was significantly lower than that of the controls until 9 months after treatment. Gain in arm muscle size and recovery from anemia were slow. The failure of the cases to catch-up in height-for-age and weight-for-age Z-scores and MUAC are related to height-for-age and weight-for-age Z-scores in both cases and controls at each measurement suggesting a consistent relationship between plasma IGF-1 levels and the indices of growth. These findings are consistent with the relationship between IGF-1, IGF-binding protein-3, and body mass index in prepubescent children reported by Mandel et al. The failure of the cases to catch-up in height-for-age and weight-for-age Z-scores and MUAC are related to height-for-age and weight-for-age Z-scores in both cases and controls at each measurement suggesting a consistent relationship between plasma IGF-1 levels and the indices of growth. These findings are consistent with the relationship between IGF-1, IGF-binding protein-3, and body mass index in prepubescent children reported by Mandel et al.

DISCUSSION

The low median admission plasma level of IGF-1 in the TDS cases was accompanied by high median serum levels of TNF and serum proteins, normal SA levels, low mean hemoglobin concentration, reduced collagen synthesis (low median plasma PICP), and impaired growth. The TDS children were severely stunted and moderately wasted. There were no significant differences in skinfold thicknesses between cases and controls throughout. Although the error in measuring skinfold thicknesses in children is high, it is possible that chronic Trichuris infection has little effect on peripheral fat. Six weeks after treatment, the cases had a normal mean weight-for-height Z-score and significant improvement in plasma levels of IGF-1 and PICP.

In the year after treatment, the cases’ increases in height velocity, which is characteristic of catch-up growth, was apparent. The cases however, remained significantly below the controls in height-for-age and weight-for-age Z-scores and HC. The mean MUAC of the cases was significantly lower than that of the controls until 9 months after treatment. Gain in arm muscle size and recovery from anemia were slow. This slow recovery in muscle protein and hemoglobin synthesis may be partly explained by several factors: the comparatively low levels of IGF-1; iron deficiency anemia and reduced muscle mass as features of the cachectic state induced by TNF; and increased iron requirements during periods of rapid growth. Plasma levels of IGF-1 were significantly related to height-for-age and weight-for-age Z-scores in both cases and controls at each measurement suggesting a consistent relationship between plasma IGF-1 levels and the indices of growth. These findings are consistent with the relationship between IGF-1, IGF-binding protein-3, and body mass index in prepubescent children reported by Mandel et al. The failure of the cases to catch-up in height-for-age and weight-for-age Z-scores and MUAC are related to height-for-age and weight-for-age Z-scores in both cases and controls at each measurement suggesting a consistent relationship between plasma IGF-1 levels and the indices of growth. These findings are consistent with the relationship between IGF-1, IGF-binding protein-3, and body mass index in prepubescent children reported by Mandel et al.

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levels of IGF-1 may indicate the extent of protein turnover and nitrogen balance. 24

Plasma PICP levels increased in the cases after treatment, exceeding the levels of their controls by the end of the first year. This may indicate increased extracellular matrix production not yet reflected in height velocity. 10 The PICP levels were not related to any of the indices of growth nor height nor weight velocities nor to any changes in them, in this group of children.

TDS children differ from severely malnourished children in that they are able to mount an acute phase inflammatory response which has a systemic as well as a mucosal component. 14 Children with protein calorie malnutrition have low serum levels of IGF-1 and low levels of TSP and SA. 24,25 They are unable to mount a normal inflammatory response. 26

Protein malnutrition impairs the ability for cytokine production. 22 High serum levels of TNF and serum proteins reflect the severity of the inflammatory process in TDS. Serum protein levels in the recovering cases were significantly higher than those of their controls. This is similar to the findings of Cooper et al. 14 The redistribution of circulating proteins with preference for those of the immune response could limit substrate for the synthesis of IGF-1 and other growth factors which require the same key amino acids for their molecular structure. TNF, by mobilizing muscle protein and stimulating the production of extracellular matrix production not yet reflected in height velocity. 10 The PICP levels were not related to any of the indices of growth nor height nor weight velocities nor to any changes in them, in this group of children.

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