

ANTHELMINTHIC TREATMENT RAISES PLASMA IRON LEVELS BUT DOES NOT DECREASE THE ACUTE-PHASE RESPONSE IN JAKARTA SCHOOL CHILDREN

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Abstract. The study was conducted to investigate the impact of intestinal helminthiasis and treatment on iron status and acute phase response (APR) among urban Indonesian primary school children, aged 8-11 years old. The prevalence of helminthiasis among these children was; *Ascaris lumbricoides*, 81.6%; *Trichuris trichiura*, 88.3%; and mixed infection of *A. lumbricoides* and *T. trichiura*, 70.0%. Of 120 children enrolled in the investigation, 59 received a single 400 mg dose of albendazole, and 61 received a placebo. Ten days following treatment, the prevalence of ascariasis and trichuriasis in the treatment group diminished to 0% and 27%, respectively, and in the placebo group to 63.9% and 68.9%. Plasma iron, hemoglobin, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), white blood cell (WBC), interleukin-1 (IL-1), interleukin-6 (IL-6) and tumor necrosis factor (TNF) concentrations were determined prior to the intervention and 10 days after. Plasma iron concentrations and WBC count rose in the treatment group ($p \leq 0.05$) when compared to baseline status. Increases in hemoglobin concentrations observed in the treatment group 10 days post-treatment were not statistically significant. CRP, IL-1, IL-6 and TNF were found to be within normal limits for both groups both before and after treatment. ESR increased significantly in both treatment and placebo groups when compared the rates measured before treatment. These findings show that treatment with albendazole is associated not only with a decreased worm burden in school children, but also a rise in plasma iron.

INTRODUCTION

It is estimated that worldwide 400 million school-age children are infected with intestinal helminths (WHO, 1995). In Indonesia, intestinal helminth infestations are known to be highly prevalent among schoolchildren (Margono, 1992). The most prevalent group of intestinal helminths are soiltransmitted such as common roundworms (*Ascaris lumbricoides*) and whipworms (*Trichuris trichiura*) (WHO, 1995). One of the effects attributed to intestinal helminthiasis infestation is impaired development of children. For example, ascariasis has been shown to delay the growth of the host in school children (Mahani, 1979). Moreover, *Ascaris* infestation interferes with food intake, absorption and retention in growing children (Mahani, 1979). Poor host nutrition utilization may be due, in part, to the nutrition requirement of the worms themselves. Twenty roundworms require approximately 2.8 g carbohydrate and 0.7 g protein per day (Hadidjaja,

1991) but other factors must be involved in the decreased utilization rates. It has also been reported that heavy infestations of *T. trichiura* cause iron deficiency anemia and growth retardation (Bundy and Cooper, 1989). *T. trichiura* is estimated to affect 800 million people globally and school aged children have the highest burden.

In some field studies, the magnitude of growth differences in dewormed children compared to helminth-infected children has been considerable (Stephenson *et al*, 1990). Deworming to expel intestinal helminths has shown to be accompanied by improved body weight, height and hemoglobin levels (Stephenson *et al*, 1989, 1993).

Iron-deficiency is one of the major nutrition-related problems among children in developing countries. This deficiency may lead to growth retardation, poor mental development, and reduced immune response (ACC Subcommittee of Nutrition, 1991). Low iron level may be due to low

intake of iron containing food, poor absorption and utilization of iron in food, and acute and chronic blood losses (INACG, 1984).

The pathogenesis of anemia caused by chronic blood loss, due to intestinal helminthiasis, is complex. It is believed that the anemia is related to reduced erythropoiesis, slightly shortened red cell survival and changes in iron metabolism. Clinically, the anemia associated with chronic disease is mild and the underlying disease usually dominates the clinical picture. Most often the anemia is present in the form of normocytic, normochromic anemia with low serum iron levels, even though the iron stores are normal or even increased (Hershko, 1992).

Different parasites have different effects on the development of children. Moreover, *Ascaris lumbricoides* infestation interferes with food intake, absorption and retention in growing children (Stephenson *et al*, 1989). It has been reported that heavy infestations of *T. trichiura* cause iron deficiency anemia and growth retardation (Stephenson *et al*, 1993).

Another impact of intestinal helminthiasis results from the intimate relationship between infection, nutritional status, and the immune system. Infectious diseases are associated with negative nitrogen balances which may precipitate overt malnutrition and immune depression (Chandra, 1980). Undernutrition and infection often coexist and may mutually augment the immune suppression and lead to immunoincompetence (Chandra, 1980). Primary immunodeficiency states are characterized by failure-to-thrive and a susceptibility to infection (Scrimshaw *et al*, 1968).

Intestinal helminthiasis may exert part of its influence on nutrition through the catabolic loss of nutrients due to the activation of the immune response. This activation is often accompanied by an acute-phase response (APR) which is an important mediator of the inflammatory response (Buck *et al*, 1994) and of disturbances of homeostasis due to infection, tissue injury, neoplastic growth or immunological disorders (Heinrich *et al*, 1990). This APR is thought to be beneficial to the injured organism, aiming at restoring physiological intactness (Heinrich *et al*, 1990).

The APR consists of a *local* reaction at the site of injury, which in turn, releases acute phase cytokines such as IL-1, IL-6 and TNF (Heinrich *et*

al, 1990). Besides local reaction, these mediators also act on specific receptors on different target cells throughout the body leading to a *systemic* reaction characterized by fever, leukocytosis, increased erythrocyte sedimentation rate (ESR), decreased serum levels of iron and zinc, and dramatic changes in the concentration of some plasma proteins such as CRP and serum amyloid A (SAA), which the two major human acute phase proteins (Kushner, 1993). It has been reported that the elevated serum levels of cachectin-TNF decreased three to six months after anthelmintic treatment of humans infested with *Schistosoma mansoni*, however, evidence is lacking for the persistence of the APR in chronic human geohelminth infection (Stephenson *et al*, 1993).

Iron deficiency has been reported to have a number of effects on immune system function (Keusch, 1989). In anemia of chronic inflammation, cytokines clearly play a major role (Kushner, 1993). The reduction in circulating iron (hypoferremia) seen during the helminth infestations may be related to demonstrated induction of ferritin production in liver cells by IL-1, and in adipocytes and muscle cells by TNF. The effect of increased ferritin synthesis is abrogate the release of iron, resulting in reduced serum iron levels (Hershko *et al*, 1992).

Previous surveys in urban Jakarta children have commonly revealed high prevalences of both intestinal helminthiasis and disturbances of iron metabolism, including anemia (Abidin *et al*, 1991; Angeles *et al*, 1993). We used the children in this setting to investigate and monitor those immediate changes in biochemical markers of iron transport and of the APR as might be expected to occur in the aftermath of the expulsion of a substantial worm burden from the host's intestine.

MATERIALS AND METHODS

The design of this study was a cross-sectional association study combined with a randomized, doubly blind, community intervention trial. Data collection was performed from December 1994 through January 1995. The study was carried out in Tanjung Priok, North Jakarta, Indonesia, at two public elementary schools (SD 01 and SD 02 Papanggo, Tanjung Priok) from a middle-to low-socioeconomic community. The environmental hygiene and sanitation in the study area were poor.

Sampled population

Three-hundred and two children from grades 2-6 (8-11 years old) were selected from among the 500 students in these two elementary schools. After obtaining parents' informed consent for the children's participation, stool samples were collected from the 302 children.

Methods

From the 269 children with positive stool samples, 160 children were selected to continue in the study. The children were selected based on their stool examination. Children were ranked based on egg-counts and children with egg-counts in descending order until 160 subjects were enrolled. Following a personal interview, 30 children were excluded because of reports of acute illness such as typhoid fever, varicella, respiratory tract infections, or fevers of unknown origin within the previous 10 days. Other exclusion criteria were moderate to severe injury or surgery during the last month, and received a deworming drug, iron tablet or corticosteroid during the last month. The remaining 130 children were separated into treatment or placebo groups based on matching the egg counts for number and type of egg. Anthropometric measurements were performed on the day before treatment, as was a first personal interview. Clinical examination was also performed on 130 children with the highest egg counts. Blood samples were taken on the day of treatment. A second personal interview, clinical examination, blood and stool samples of these children were obtained at the end of the study 10 days after treatment. Ten children had to be excluded from the study because they suffered from acute infectious diseases at the time of the first or second blood collection. As a result, we studied 120 individuals: 59 subjects (35 males and 24 females) in the treatment group and 61 subjects (26 males and 35 females) in the placebo group.

Ethical considerations in this study were based on the Council for International Organizations of Medical Sciences (CIOMS) guidelines (CIOMS, 1991). The research proposal was approved by the Committee on Health Research Ethics, Health Research and Development, Department of Health Republic of Indonesia and US Naval Medical Research Unit No.2 (NAMRU-2) Committee for the Protection of Human Subjects (CPHS).

Stool examinations for intestinal helminths were performed on the day of the excretion using a modified Kato technique (Suzuki, 1977). Intensity of infection was expressed as eggs per gram of feces (epg). Percentage of egg reduction rates from exam 1 to exam 2 were also calculated from the arithmetic and geometric mean egg counts utilizing the formula:

$$\% \text{ egg reduction} = \frac{(\text{initial epg} - \text{final epg})}{\text{initial epg}} \times 100.$$

All assessment of stool samples were analyzed at the Department of Parasitology, Medical Faculty, University of Indonesia and US Naval Medical Research Unit No.2 (NAMRU-2).

Personal interviews were performed utilizing prepared questionnaire which consisted of 25 questions divided into 5 main sections: 1) identification of the children; 2) personal hygiene practices and sanitation; 3) morbidity history; 4) drugs or supplement usage and 5) present and recent illness.

Subjects were weighed without shoes using an electronic, platform model weighing scale (SECA 770 alpha; SECA, Hamburg, Germany). The weight was recorded to the nearest 0.1 kg. Height was measured to the nearest 0.1 cm using a microtoise. Z-scores of the indicators Weight For Age (WFA), Weight For Height (WFH) and Height For Age (HFA) were calculated by using the National Center for Health Statistics (NCHS) reference data to correct for effects of sex and age on growth (NCHS, 1977). Clinical examination was done during the data collection by a physician from the Medical Faculty, University of Indonesia to screen whether the subjects suffered from diseases.

Fasting blood samples (15 ml) were collected via venipuncture before breakfast. These blood samples were used to determine plasma iron concentration, hemoglobin, Erythrocyte Sedimentation Rate (ESR), White Blood Cell (WBC), C-Reactive Protein (CRP), Interleukin-1 (IL-1), Interleukin-6 (IL-6) and Tumor Necrosis Factor (TNF). Plasma iron concentration was measured according to the Ferrozine-method using a commercial kit (Merck, Darmstadt, Germany). The cut-off point for normal plasma iron is $> 59 \mu\text{g/dl}$ (male) and $> 37 \mu\text{g/dl}$ (female) (Manual of Iron-Ferrozine Method, 1994). We obtained assays on almost all samples obtained, but 24 samples are missing due to hemolysis of blood samples during the laboratory analysis. Hemoglobin and WBC were deter-

mined by using quantify buffy coat cell count (QBC) autoread hematology analyzer (Becton Dickinson, New Jersey, USA). The criterion for anemia in this study was Hb level < 120 g/l. The normal value for the white blood cell count is $4.3\text{--}10.0 \times 10^9$ cells/l. ESR was measured according to the Wintrobe technique using a Becton-Dickinson Seditube (New Jersey, USA). Normal values for ESR range from 0 to 25 mm/hour. CRP was determined according to the Turbidimetric method (Metzmann, 1985). The cut-off point for normal CRP is 0.5 ng/dl. IL-1 was measured according to ELISA technique using a commercial kit (Quantikine, R&D Systems, Minneapolis, MI). IL-6 was measured by a bioassay technique using the IL-6 dependent cell line B9 (Coligan *et al*, 1991). TNF was determined by a cytotoxicity assay using WEHI-164 target cells (Coligan *et al*, 1991). The cut-off criterion for normal IL-1, IL-6 and TNF were: <0.3 pg/ml, <1U/ml, and <1U/ml, respectively.

Based on World Health Organization recommendation (WHO, 1995), the treatment group received a single dose of 400 mg albendazole (SmithKline Beecham, Ltd, Brentford, Middle Essex, UK). The control group received the placebo which had a similar appearance to the deworming drug prepared by PT. Pharos under license from SmithKline Beecham, Ltd. The drug and placebo were coded and blinded to surveyors and subjects. The assigned medication was taken by the subjects in the presence of the surveyors. The placebo groups with worms received the deworming drug at the end of the study.

Statistical procedures

Data were stored using Epi-info (version 6.0) and analyzed with SPSS-PC+ and Epi-Info (version 6.0). The statistical test used was MANOVA repeated-measures design with treatment (deworming drug and placebo) as a between-subjects factor and time (before *vs* after) as a within-subject factor. Chi-square test and Fischer test were used for association, and McNemar's test was used to identify the changes in prevalence, ANOVA and paired *t* tests were used to assess the differences between and within groups. Pearson's correlation was used to test correlation. When values were not normally distributed, as for hemoglobin level, differences within groups were tested by using Wilcoxon's matched-pairs signed-rank test and differences between groups were tested by using the Mann-

Whitney test. Egg counts were logarithms transformed with the $n+1$ transformation before applying parametric tests (Sokal and Rohlf, 1969).

RESULTS

Two-hundred and sixty-nine fecal specimens were found to have helminths ova for an overall prevalence of intestinal helminthiasis of 89%.

The mean (\pm SD) age of the albendazole-treated and placebo groups were 112 ± 12.0 and 111 ± 11.5 mo, respectively. Because of the design of the experiment all children were infected with helminths; 81.6% of the children were infected with *A. lumbricoides*, and 88.3% with *T. trichiura*. At the start of the study, no significant differences existed in the prevalence or intensity of the intestinal helminthiasis between the placebo and treatment of children selected for treatment. The changes in prevalence of infection were significantly greater with albendazole than in the placebo group (Table 1). With respect to intensity, *A. lumbricoides* eggs per gram feces were significantly decreased both in the treatment and placebo groups. The egg reduction in the treatment group was 100% and showed a significantly higher reduction than in the placebo group (Table 2). There was a significant difference both in the treatment and placebo group for *T. trichiura* eggs before and after 10 days after treatment, however the before-after changes were not significantly different between the two groups (Table 2). The intensity of *A. lumbricoides* infection as shown in Fig 1; the range of egg counts for both the treatment and placebo groups was 0-30,000 eggs/gram feces at the start of the study. After anthelmintic treatment, all subjects in the treatment group were negative for eggs of *A. lumbricoides* ova. Eggs per gram feces of *T. trichiura* infection in both groups were ranged between 0-1,500, both before and after treatment. However, 10 days after treatment, the percentage of subjects who were negative for *T. trichiura* eggs in treatment group (55%) was greater than in the placebo group (30%) (Fig 1).

The mean values of Z-score of WFA, WFH and HFA were -1.48 ± 0.78 , -0.81 ± 0.95 and -1.40 ± 0.83 , respectively. The rate of children with Z-score of WFA, WFH and HFA below -2 were 24.2%, 6.7% and 19.2%, respectively. There were no significant differences on nutritional status (Z-score of HFA)

Table 1

Prevalence of intestinal helminthiasis in 120 children before and 10 days after treatment with albendazole (n=59) or placebo (n=61).

Prevalence of intestinal helminthiasis	Before treatment		After treatment	
	(%)	(n)	(%)	(n)
<i>Ascaris lumbricoides</i>:				
Treatment group ^{a,b}	81.4	48	0	0
Placebo group ^b	82.0	50	63.9	39
<i>Trichuris trichiura</i>:				
Treatment group ^{a,b}	89.8	53	45.8	27
Placebo group ^b	86.9	53	68.9	42

^aChanges in prevalence significantly greater in the treatment group than in the placebo group:

$p \leq 0.001$ (chi-square)

^bSignificant difference before and after treatment (within group), $p < 0.05$ (McNemar test)

Table 2

Intestinal helminthiasis intensity expressed as means of eggs/g before and 10 days after treatment with albendazole (n=59) or placebo (n=61).

	Arithmetic mean				% egg	Geometric mean				% egg
	Before	(n)	After	(n)	reduction	Before	(n)	After	(n)	reduction
<i>Ascaris lumbricoides:</i>										
Treatment group ^{a,b}	2539	48	0	48	100	72	48	0	48	100
Placebo group b	2410	50	1893	39	21	78	50	60	39	23
<i>Trichuris trichiura:</i>										
Treatment group ^b	163	53	116	27	29	25	53	16	27	36
Placebo group ^b	161	53	123	42	24	23	53	17	42	26

^aSignificantly greater than the value of the placebo group after treatment (Geometric mean):

$p \leq 0.001$ (*t*-test)

^bSignificant difference before and after treatment (Geometric mean), $p < 0.05$ (Paired *t*-test)

between the prevalence of those two types of helminths. There was also no significant correlation between the Z-score of HFA and the mean values of *A.lumbricoides* and *T.trichiura* egg per gram feces. Furthermore, there was no significant correlation between the markers of APR (WBC and ESR) and the Z-score of HFA.

In the study population, 30% of the subjects were anemic (Hb < 120g/l) and 21.6% had plasma iron levels below normal. There was an increase in hemoglobin level after treatment in the treatment group although this difference was not significant (Table 3). The increase of plasma iron was significant in both groups, but the treatment group increased significantly more than the placebo group

(Table 3). Ten days after treatment, the before-after differences in plasma iron for the treatment group was significantly higher than those for the placebo group ($p = 0.025$) whereas the difference within the treatment group was +6.51 $\mu\text{g/dl}$ and the difference in the placebo group was -9.46 $\mu\text{g/dl}$ (Table 3).

The acute phase response markers such as CRP, IL-1, IL-6 and TNF in the treatment and placebo groups showed normal values both before and 10 days after intervention. ESR showed a significant increase in both groups (Table 4). Within-group increases in white blood cell count was significant only in the treatment group (Table 4). The interaction of both factors group (treatment vs placebo)

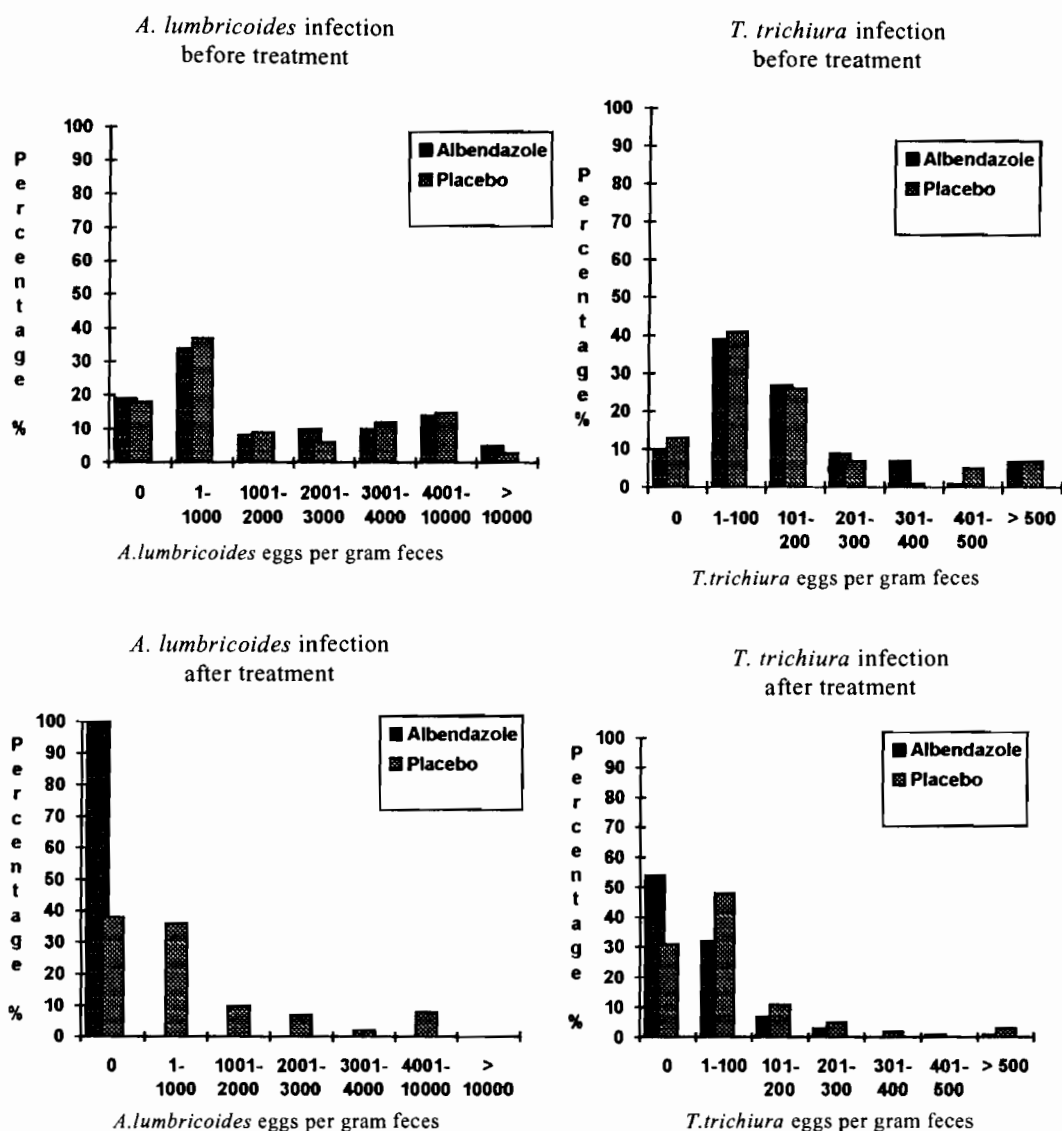


Fig 1-Distribution of intensity of *A. lumbricoides* and *T. trichiura* infection in albendazole and placebo groups before and 10 days after treatment.

and timing (before vs after) regarding the means of ESR and WBC were statistically significant.

DISCUSSION

The high prevalence of intestinal helminthiasis in the children studied confirms its importance as public health problem in Indonesia. Treatment of

soil-transmitted helminth infections with deworming drug is a recommended measure of control (Margono, 1992; Abidin *et al*, 1991). In this study, treatment with a single dose of albendazole (Smith Kline and French Laboratories (Aust) LTD, 1979) showed 100% effectiveness against *A. lumbricoides* 10 days after treatment, but only 54.2% against *T. trichiura* infection. These results are similar to reports from Indonesia (Abidin *et al*, 1991) and other countries (WHO, 1995). Of low cure rates for

Table 3

Plasma iron status of treatment and placebo groups before and 10 days after treatment^a

	(n)	Before	After	Difference
Treatment group:				
Plasma iron (µg/dl)	53	72.19±22.27	78.65±29.69	+6.51±31.12 ^{b,c}
Hemoglobin(g/dl)	40	12.54±1.13	13.27±2.58	+0.73±2.70
Placebo group:				
Plasma iron (µg/dl)	53	86.33±35.14	76.87±31.73	-9.46±33.42 ^b
Hemoglobin (g/dl)	35	12.78±1.17	12.63±2.39	-0.15±2.54

^amean±SD^bSignificant difference between before and after treatment (within group change), $p < 0.05$ (Paired *t*-test)^cSignificantly higher than the related value of the placebo group, $p = 0.012$ (*t*-test)

Table 4

Acute phase response of treatment and placebo groups before and 10 days after treatment.

Acute phase response	(n)	Before treatment (mean±SD)	After treatment (mean±SD)	Difference (mean±SD)
White blood cell ($\times 10^9$ cells/l):^b				
Treatment group ^a	29	6.4±1.7	7.4±2.3	1.0±2.1
Placebo group ^a	20	6.6±2.4	7.7±2.2	1.1±2.5
Erythrocyte sedimentation rate (mm/h):^b				
Treatment group ^a	15	11.4±9.0	17.3±7.9	5.9±8.9
Placebo group ^a	19	10.8±7.0	17.8±9.5	7.0±8.5

^aSignificant difference before and after treatment (within-group change), $p < 0.05$ (Wilcoxon test)^bSignificant difference of factors group (treatment vs placebo) and timing (before vs after), $p < 0.05$ (MANOVA test)

trichiuriasis with a 400 mg albendazole single-dose treatment. As a result, this study supports the observations of Margono *et al* (1994) that repeated treatment with albendazole may be necessary to eradicate *T. trichiura* infection.

After anthelmintic treatment, the percentage of subjects in the treatment group negative for *T. trichiura* eggs was higher than in the placebo group (Fig 1). The unexpected decreases of prevalence, and egg reduction in the placebo group may be due to parents' reaction to reading the consent form was to make sure that their own children were covered, namely, by purchasing and administering anthelmintics on their own.

Despite the high prevalence of trichiuriasis and ascariasis in children in this study, the average worm burden was generally low (Table 2). Data from studies around the world on *A. lumbricoides* have shown that if the prevalence is greater than 60%, then the mean worm burden may vary greatly (Hall, 1993). Considering that the nutritional impact of helminth infection on population groups is mainly related to the severity of the infection and to a lesser extent to the prevalence (Stephenson *et al*, 1989), it has to be acknowledged that prevalence alone is a relatively insensitive indicator to predict potential nutritional problems due to intestinal worm infestations as shown in this report and by others (Solomons and Scott, 1994).

There was no significant correlation between HFA Z-score and worm burden. This suggests that worm intensity was not directly related to decreased a child's height. Traditionally, it is believed that low HFA and WFA is caused by a lack of protein and/or energy and is therefore called protein-energy malnutrition (PEM). A large number of *A. lumbricoides* would compete for protein and energy in the intestinal tract of a small child. A study by Stephenson *et al* (1989) showed that during the 6 months after a single dose of albendazole improves the growth of Kenyan school children with hookworm, *Trichuris* and *A. lumbricoides* infection. It has been shown that worm infection can depress growth, physical fitness, physical activity and cognitive performance via two pathways, both of which have depressed appetite as a central feature (Stephenson *et al*, 1993). The enhancing effects of deworming treatment on growth of infected children with parasites has been reported elsewhere (Stephenson *et al*, 1989, 1993; Adams *et al*, 1994). However, in this study the low intensity worm-burden may have had a limited effect on child growth and it is internationally acknowledged that stunting is not an appropriate indicator of malnutrition because infectious diseases may also contribute directly to growth retardation of children (WHO, 1986). Therefore, it is not possible to relate height solely to intestinal helminthiasis because several uncontrolled additional factors may have contributed to the linear growth rate of the studied children.

The results of this study showed that the anthelmintic treatment caused an increase in plasma iron levels. In addition after 10 days treatment that hemoglobin level increased in the treatment group, whereas it decreased in the placebo group. Anemia is an established public health problem among urban, Indonesian children (Hershko, 1992; Angeles *et al*, 1993) and our cross-sectional findings confirm this situation. Although we measured hemoglobin over a 10-day interval, the well-known kinetics of red cell turnover would not have permitted any observation of a treatment effect in only 1/12th of the red-cell lifespan even if an eventual hematologic effect were to have been produced. In this study, increased iron transport secondary to helminth treatment could have been caused by improved utilization of ingested iron because treatment with albendazole is highly active against eggs, the larval and adult stages. *T. trichiura* infestation involves blood loss to the host using its

mouth spear to cut and slash tissues and blood vessels. It then enters the open blood vessel or the shredded tissue area and sucks the blood using its muscular esophagus. This causes 6 to 10 times less blood loss per worm compared with *N. americanus* and 30 to 50 times less blood loss compared with *Ancylostoma duodenale* (Taren and Crompton, 1989). One *T. trichiura* adult worm (female) can produce about 5,000 eggs per day (Margono, 1988). So, if treatment with albendazole were effective against the adult worm of *T. trichiura*, the iron status of the children should improve. Hypoferremia seen during the APR may be related to the demonstrated induction of ferritin in liver cells by IL-1, and in adipocytes and muscle cells by TNF α . The result of increased ferritin synthesis is a block in iron release, resulting in reduced serum iron levels (Hershko, 1992). In this study, level of circulating of IL-1 and TNF were normal. Therefore they were not likely to inhibit in increased plasma iron levels an indicator that APR is involved in the increase of iron status is that also an increase in iron status was found in the ascariasis group although this worm does not cause bleeding. Assessment of serum ferritin levels (which were not measured in this study) gives information on iron storage as the initial sign of anemia due to the parasitic infection (Cook, 1990). Transferrin saturation measurement (also not measured in this study) would have been helpful since it decreases in parallel with the plasma iron concentration when the former is based on nutritional deprivation (INACG, 1984).

IL-1, IL-6 and TNF levels of individuals in this study were normal, although the children suffered from intestinal helminthiasis. A study showed that children with *T. trichiura* dysentery had increased TNF production in their colon by assessment of multiple biopsy in the lamina propria of the colon (Macdonald *et al*, 1994). *Trichuris* dysentery syndrome is a disease resulting from heavy infection of the large intestine by *T. trichiura* which resulted in children who have chronic dysentery and frequently are severely growth-impaired (Bundy and Cooper, 1989). It has been observed that cytokines like IL-1, IL-6 and TNF are induced by factors generated at the site of tissue injury (Raynes, 1994). The study showed that after 6 to 12 hours the parasitic nematode *Heligmosomoides polygyrus* infection resulted in marked elevations of particular cytokines (IL-3, IL-5 and IL-9) which were detected in the Payer's patch (Svetic *et al*, 1993). This change in cytokine gene expression occurred in the spleen which sug-

gests that the immune response remains localized to the gut-associated lymphoid tissues and that cells in this region probably play the primary role in promoting the observed increases in serum IgE and blood eosinophil levels (Raynes, 1994). Normal levels of the cytokines observed in the serum in this study suggest that although there may have been elevated levels locally there were no significant cytokine concentrations measured in the peripheral system. Systemic production of cytokines, especially TNF, is common with severe illness due to hypovolemic shock, sepsis, trauma and tissue injury (Roubenoff and Rall, 1993). It is likely that most of the intestinal helminthiasis had developed over years and therefore represented *chronic* infestation. Thus the APR did not play a major role in our study population.

Like the cytokines, C-reactive protein showed normal values in all subjects. Thus may be because the intestinal helminthiasis was chronic. We know that the level of CRP may increase (1,000) fold greater than normal in some severely infected individuals (Kushner, 1993). After 10 days treatment, ESR within each group increased significantly (Table 4), but the changes were within the normal range and not significantly different between treatment and placebo groups. Elevation of this marker represents a systemic reaction during acute intestinal helminthiasis but it is not specific in the case of chronic infections (Wintrobe *et al*, 1974). This means the elevation of this marker may be a sign of inapparent infection (Solomons *et al*, 1994) or the episode of acute illness (Widmann, 1983) within 10 days after treatment.

A primary interest of this study was to provide insights into the question of whether intestinal helminthiasis, causes sufficient inflammation to provoke the acute-phase response in children. Clearly, the most direct and straight-forward way to assess this question would be by manipulating a single variable. *Prospective infection* of individuals with *Ascaris* or *Trichuris* ova and measurement of APR might be conclusive but ethical constraints, especially if the "volunteers" were to be drawn from growing healthy children, would prevent approval of this protocol. The alternative and more ethically viable intervention is to find children who already have intestinal worm burdens, and to monitor the changes in APR markers in association with the eradication of the worm infection. This was the approach used here. However, for such an inquiry to be viable, not only must the population have

intestinal parasites (as ours did), but also abnormalities in the APR markers prior to the treatment. It was clear to us when the results of the baseline sampling of CRP, WBC, ESR, and cytokines had been completed, that we had little or no room in the distribution to detect a significant *downward* correction in these markers, they were tightly distributed within the non-pathological range.

Before concluding, however, that the hypotheses relating helminthiasis to the APR is invalid, the absolute level of the worm burden must be considered. The <50 to 30,000 eggs/g for *A. lumbricoides* should correspond to 1 to 10 adult worms (Pesigan *et al*, 1958). Similarly, for fecal ova concentrations in the < 50 to 1,500 per gram range for *T. trichiura* should correspond to 1 to 15 adult worms (Pesigan *et al*, 1958). Human experience more intense infections involving hundreds of helminths of both species (Bundy, 1994). Although hygienic conditions were judged to be poor in Tanjung Priok, transmission of geohelminths is modest compared to that in more rural areas of Sumatra or Irian Jaya. An adequate test of the association can only come in the context of populations with elevated APR markers and with much more substantial worm burdens.

Interest has arisen in the roles played by intestinal worms, other organisms and inert antigens in the concept of chronic immunostimulation in free-living children (Solomons *et al*, 1993). Ruz *et al* (1995) found 36% of a sample of anemic children from across the Republic of Guatemala to have an elevation of the WBC or ESR or both in the absence of any clinical manifestations of acute infection. The finding elevation of these variables in 17% of preschool children from an underprivileged periurban settlement of Guatemala City by Valdez *et al* (1995) confirms this phenomenon. Thus, the absence of any background evidence of activation of the APR in this present population is worthy of comment. Like the Guatemalan poor, these children were free of clinical findings of infection, but unlike their Central American counterparts not a single child had abnormalities in the markers of the APR. This could be explained by some quantitative degree in the density of environmental microbes thought to produce the chronic immunostimulation (Solomons *et al*, 1993).

Acknowledging that adequate helminthiasis treatment can improve plasma iron level in school children, the question remains whether deworming

campaigns are the most appropriate intervention strategy in chronic helminthiasis for alleviating iron deficiency anemia especially when the underlying causes, lack of hygiene and sanitation are not addressed simultaneously and reinfection is very likely. In this study, the lack of evidence of a systemic APR indicates an immunological adaptation to the helminthiasis that may well deteriorate if repeated anthelmintic treatment is given. If underlying causes are not controlled at the same time by intervention measures, the repeated APR responses that might ensure with re-injection may be more deleterious to the host and should be evaluated. Nonetheless, in cases of chronic helminthiasis, and improvement of plasma iron concentration can be reached by treatment, even in very low-intensity worm burdens, such as those in urban Jakarta school children. Only more prolonged observations with a sustained deworming will answer the question of the magnitude of the contribution of anthelmintic therapy to the reduction of iron deficiency and anemia, immunocompetence and other nutrition related growth of development characteristics in this age-group.

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REFERENCES

Abidin SAN, Ismid IS, Margono SS. The prevalence and

intensity of *Ascaris* and *Trichuris* infection with different treatment schedules. Progress report of Department of Parasitology, Faculty of Medicine, University of Indonesia, Jakarta, 1991.

ACC / Subcommittee of Nutrition. Controlling micronutrient deficiencies. Some for improving nutrition in the 1990's In: Supplement to option SCN News, 1991;7:17.

Adams EJ, Stephenson LS, Latham MC, Kinoti SN. Physical activity and growth of Kenyan school children with hookworm, *Trichuris trichiura* and *Ascaris lumbricoides* infections are improved after treatment with albendazole. *J Nutr* 1994; 124, 1119-204.

Angeles TI, Schultink WJ, Matulessi P, Gross R, Sastroamidjojo S. Decreased rate of stunting among anemic Indonesian preschool children through iron supplementation. *Am J Clin Nutr* 1993;58:339-42.

Buck C, Bundschu J, Gallati H, Bartmann P, Pohlandt F. Interleukin-6: A sensitive parameter for the early diagnosis of neonatal bacterial infection. *Pediatrics* 1994;93:54-8.

Bundy D, Cooper E. *Trichuris* and trichuriasis in humans. *Advances Parasitol* 1989;28:107-73.

Bundy DAP. The global burden of intestinal nematode disease. *Trans R Soc Trop Med Hyg* 1994;88:259-61.

Chandra RK. Immunology of nutritional disorders. London: Edward Arnold, 1980:2-3.

Coligan JE, Kruisbeek AM, Margulies DH, Shevach EM, Strober, W. Current protocols in immunology. New York: Greene Publishing Associated and Wiled Interscience 1991:6.6.1-5.

Cook JD. Adaptation in iron metabolism. *Am J Clin Nutr* 1990;51:301-8.

Council for International Organizations of Medical Sciences (CIOMS). International guidelines for ethical review of epidemiological studies, Geneva, 1991.

Hadidjaja P. Pidato pengukuhan guru besar tetap. Department of Parasitology, Faculty of Medicine, University of Indonesia, Jakarta, 1991.

Hall A. Intestinal parasitic worms and the growth of children *Trans R Soc Trop Med Hyg*, 1993; 87: 241-2.

Heinrich PC, Catell JV, Andus T. Review Article: Interleukin-6 and the acute phase response. *Biochem J* 1990; 265: 621-36.

Hershko C. Iron and infection. In: Fomon SJ, Zlotkin S, eds. Nutritional anemia: Nestlé Nutrition Workshop Series. New York: Raven Press, 1992.

International Nutritional Anemia Consultative Group

- (INACG). Iron deficiency in women. Washington, DC, 1984:31.
- Keusch GT. Immunity and infection in iron deficiency. In: Enwonwu CO, ed. Functional significance of iron deficiency. Third Annual Nutrition Workshop. Meharry Medical College, USA, 1989: 25-9.
- Kushner I. Regulation of the acute phase response by cytokines. *Perspect Biol Med*, 1993; 36: 4.
- MacDonald TT, Spencer J, Murch SH, *et al.* Immunoepidemiology of intestinal helminthic infections. Mucosal macrophages and cytokine production in the colon of children with *Trichuris trichiura* dysentery. *Trans R Soc Trop Med Hyg* 1994; 88: 265-8.
- Mahani BZ. The influence of ascariasis infestation on some aspects of nutritional status of school children between seven to nine years. Faculty of Medicine, University of Indonesia, Jakarta, 1979. Master Thesis.
- Manual of Iron-Ferrozin Method. E Merck, Postfach, Darmstadt, 1994.
- Margono SS. Helminthologi. In: Gandahusada S, Pribadi W, Ilahude HD, eds. Parasitologi Kedokteran. Balai Penerbit Fakultas Kedokteran Universitas Indonesia Jakarta, 1988.
- Margono SS. Improving knowledge, attitude and practice of the community in the field of parasitology with special attention to environmental problems. *Med J UI* 1992; 1:123-9.
- Margono SS, Abidin SAN, Mahfudin H, Ismid IS. Pengobatan cacing-cacing yang ditularkan melalui tanah khususnya *Trichuris trichiura* dengan albendazol dan mebendazol. *Majalah Parasitol Indon* 1994; 7:1-7.
- Metzmann E. Protein quantitation on both branches of the Heidelberg curve by monitoring the kinetic of immunoprecipitation. *Behring Inst Mitteilungen* 1985; 78:167-75.
- National Center for Health Statistics (NCHS). NCHS growth curves for children, birth-18 years. Washington DC: US Department of Health, Education and Welfare, 1977.
- Pesigan TP, Faraog NG, Hairston JJ, *et al.* Studies on *Schistosoma japonicum* infection in the Philippines. General consideration and epidemiology. *Bull WHO* 1958; 18:345-455.
- Raynes JG. The acute phase response. *Biochem Soc Trans* 1994; 22: 69-74.
- Roubenoff R, Rall LC. Humoral mediation of changing body composition during aging and chronic inflammation. *Nutr Rev* 1993; 51:1-11.
- Ruz M, Solomons NW, Mejia LA, Chew F. Alteration of circulating micronutrients with overt and occult infections in anemic Guatemalan preschool children. *Int J Food Sci Nutr* 1995; 46:267-65.
- Scrimshaw NS, Taylor CE, Gordon JE. Interaction of nutrition and infection. Geneva: World Health Organization. Monograph Series 57, 1968.
- Smith Kline and French Laboratories (Aust). 'Albendazole' clinical trial submission to Indonesia. Summary and Protocol No. 206, 1979.
- Sokal RR, Rohlf FJ. Biometry. San Francisco: WH Freeman, 1969.
- Solomons NW, Mazariegos M, Brown KH, Klasing K. The under-privileged, developing country child: Environmental contamination and growth revisited. *Nutr Rev* 1993; 51: 327-32.
- Solomons NW, Mazariegos M, Vettorazzi, C, *et al.* Growth faltering, protein metabolism and stimulation: new speculations on the nature of the relationship with notes from observations and analyses in Guatemala. In: Fjeld C, ed. Co-ordinated Research Programme on Application of Stable Isotope Tracer Methods to Studies of Amino Acids, Proteins and Energy in Malnourished Populations of Developing Countries. Report on the First Research Co-ordination Meeting. NAHRES-21, IAEA, Vienna, International Atomic Energy Agency, 1994: 115-26.
- Solomons NW, Scott ME. Nutritional status of host populations and parasitic infection. In: Scott ME, Smith G, eds. Ecological Epidemiology of Infectious and Parasitic Disease. Orlando: Academic Press, 1994: 104-14.
- Stephenson LS, Latham MC, Kurz KM, Kinoti SN, Brigham H. Treatment with a single dose of albendazole improves growth of Kenyan school children with hookworm, *Trichuris trichiura* and *Ascaris lumbricoides* infection. *Am J Trop Med Hyg* 1989; 41:78-87.
- Stephenson LS, Latham MC, Kinoti SN, Kurz KM, Brigham H. Improvement in physical fitness of Kenyan school boys infected with hookworm, *Trichuris trichiura* and *Ascaris lumbricoides* following a single dose of albendazole. *Trans R Soc Trop Med Hyg*, 1990; 84:277-82.
- Stephenson LS, Latham MC, Adams EJ, Kinoti SN, Pertet A. Physical fitness, growth and appetite of Kenyan school boys with hookworm, *Trichuris trichiura* and *Ascaris lumbricoides* infection are improved four months after a single dose of albendazole. *J Nutr* 1993; 123:1036-46.
- Suzuki N. Cellophane thick smear. Proceeding, First Conference. Asian Parasite Control Organization, Japan, 1977.

- Svetic A, Madden KB, Zhou X, *et al.* A primary intestinal helminthic infection induces a gut associated elevation of Th2-associated cytokine and IL-3. *J Immunol* 1993;150:3434-41.
- Taren DL, Crompton DWT. Nutritional interaction during paratism. *Am J Clin Nutr* 1989;8:227-38.
- Valdez C, Mazariegos M, Grazioso CF, Solomons NW. Prevalence of apparent and inapparent infections in preschool children in a peri-urban community of Guatemala. International Child Health: A digest of current information, 1995 (in press).
- Widmann FK. Clinical interpretation of laboratory tests. Philadelphia : FA Davies Comp, 1983.
- Wintrobe MM, Lee RG, Boggs DR, Bithell CT, Athens JW, Foerster J. Clinical hematology. 7th ed. London: Henry Kimpton Publishers, 1974: 125-8.
- World Health Organization Working Group. Use and interpretation of anthropometric indicators of nutritional status. *Bull WHO* 1986;64:929-41.
- World Health Organization. Health of school children. Treatment of intestinal helminths and schistosomiasis. Geneva: World Health Organization, 1995.