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Current prevalence of intestinal parasitic infections and their impact on hematological and nutritional status among Karen hill tribe children in Omkoi District, Chiang Mai Province, Thailand

Jintana Yanola[a], Woottichai Nachaiwieng[b], Suwit Duangmano[a], Mujalin Prasannarong[c], Pradya Somboon[d], Sakorn Pornprasert[a,*]

[a] Department of Medical Technology, Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai, Thailand
[b] Department of Public Health, School of Health Science, Mae Fah Luang University, Chiang Rai, Thailand
[c] Department of Physical Therapy, Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai, Thailand
[d] Department of Parasitology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand

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ABSTRACT

Intestinal parasitic infection represents a substantial problem for children living in rural or limited resources areas and significantly relates to anemia and nutritional status. This study aimed to determine the current prevalence of intestinal parasitic infections among school-age children of Karen hill tribe population in Omkoi District, Chiang Mai Province, Thailand and assess the impact of intestinal parasitic infection on hematological and nutritional status in those children. A total of 375 Karen hill tribe children, 6–14 years of age, in Omkoi District were randomly selected to participate in this study. Stool samples were collected and examined for intestinal parasitic infection through formalin-ether concentration method. Blood samples were collected for hematological and iron analysis. The overall prevalence of intestinal parasitic infection was 47.7% (179/375), with single infections (29.3%) and polyparatism (18.4%). The most common pathogenic parasite was *Trichuris trichiura* (16.0%), followed by *Ascaris lumbricoides* (13%) and *Giardia lamblia* (3.5%). In addition, non-pathogenic amoeba, *Entamoeba coli* was observed with a high prevalence rate (31.2%). Anemia and eosinophilia prevalence were 6.40% (24/375) and 74.7% (280/375), respectively. Eosinophilia was significantly more prevalent in children with intestinal parasitic infection compared to uninfected children. Among 249 children, 13.7% were iron deficiency, 9.6% were thalassemia and hemoglobinophathy and 8% were G-6-PD deficiency. A high prevalence infection rate was significantly associated with eosinophilia, but independently related to anemia and iron deficiency. Intestinal parasitic infections are endemic in school-age children of Karen hill tribe population in Omkoi District. These data highlight the need for an integrated approach to control transmission of intestinal parasites and improve the health and sanitation status of Karen hill tribe children in Thailand.

1. Introduction

Intestinal parasitic infections, including soil-transmitted helminths (STHs), remain important public health issues in low- and middle-income countries in the tropics and subtropics (Pullan et al., 2014; World Health Organization, 2017). Globally, approximately 77% of population harbors at least one species of intestinal parasites (Pullan and Brooker, 2012; Pullan et al., 2014). Estimated one billion school-age children live in endemic area of STHs with 69% of these children living in Asia and they bear the greatest burden in terms of morbidity and mortality (Pullan and Brooker, 2012; Pullan et al., 2014; Kunwar et al., 2016). In children, intestinal parasitic infections, especially helminthiasis, may cause a number of negative health outcomes such as malnutrition, anemia, impaired growth and cognitive development (Oninla et al., 2010; Fürst et al., 2012; Sayasone et al., 2015). Additionally, they may contribute substantially to increase the burden of poverty, impaired mental and educational development in children and damage economic productivity (Oninla et al., 2010; Fürst et al., 2012; Sayasone et al., 2015; Hotez et al., 2014). The World Health Assembly set a global target of administering chemotherapy to at least 75% of school-age children in endemic area of STHs and eliminating morbidity due to STHs in children by 2020 (World Health Organization, 2011; World Health Organization, 2017).

In Thailand, intestinal parasitic infections have been recognized as

* Corresponding author at: Department of Medical Technology, Faculty of Associated Medical Sciences, Chiang Mai University, 110 Intawaroros Road, Chiang Mai, 50200, Thailand.
E-mail address: sakorn.pornprasert@cmu.ac.th (S. Pornprasert).

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one of major public health in children, especially hill tribe children who live in highlands with insufficient public utilities (Piangjai et al., 2003; Saksirisampant et al., 2004; Maneeboonyang et al., 2008; Apidechkul, 2015). Chiang Mai Province is located in the North region of Thailand and surrounded by the mountains which are home of hill tribe villagers. In previous studies, intestinal parasitic infections were highly endemic in the Karen hill tribe children in Mae Chame District, Chiang Mai Province with prevalence infection rates of 42.1% (Piangjai et al., 2003; Saksirisampant et al., 2004), which were higher than infection rates of children in lowlands (4.2–12.6%) (Saksirisampant et al., 2006; Ngrenngarmlert et al., 2007). Infection with STHs, including *Ascaris lumbricoides*, hookworm and *Trichuris trichiura* showed highest prevalence rates in these Karen hill tribe children (Piangjai et al., 2003; Saksirisampant et al., 2004). However, the current intestinal parasitic infections status of this population in Omkoi District remains unknown. In addition to intestinal parasitic infections, hematological disorders were also another health problem emerging among Karen hill tribe children in Chiang Mai including anemia, thalassemia, iron deficiency, and glucose-6-phosphate dehydrogenase (G-6-PD) deficiency (Yanola et al., 2014). STHs infections in heavy intensity are the major causes of morbidity including intestinal bleeding, impaired iron status, anemia, malabsorption and diarrhea in human (Hall et al., 2008; World Health Organization, 2011). Hookworm infection is well documented in relation to the effect and risk factor of anemia in association with intensity of infection (Olsen et al., 1998; Brooker et al., 1999). However, there are few published studies describing the relationship between intestinal parasitic infections with hematological disorders and malnutrition in Karen hill tribe children. Therefore, this study aimed to assess the current prevalence of intestinal parasitic infections in school-age children of Karen hill tribe population in Omkoi District, Chiang Mai Province and determine the impact of intestinal parasitic infections on hematological and nutritional parameters. Investigation of this issue is particularly relevant for the design of integrated control strategies to reduce infection rate and anemia, including anthelmintic treatment, micronutrient supplementation, and health and hygiene education through school health programs.

2. Materials and methods

2.1. Study site and subject

The study was carried out during 2015–2016 in three sub-districts of Omkoi District, located about 179 kilometers southwest of Chiang Mai city, Chiang Mai Province, Thailand. The participants were Karen hill-tribe children aged 6–14 years old, attending the five government schools in the community of 1. Ban Yang Poa, Omkoi sub-district, 2. Chumchon Ban Mai, Mae Tuen sub-district, 3. Ban Autoom, 4. Ban Hang Luang 5. Ban Bai Na, Na Kian sub-district. This study was approved by the Ethics Committee of the Faculty of Associated Medical Sciences, Chiang Mai University (Reference No 053E/57). The Karen hilltribe children were enrolled if their parents or legal guardians provided written informed consent. Some illiterate parents or legal guardians signed by means of a thumb print for which a specific section on the informed consent form was created and approved by the respective ethical committees. Treatment was provided for children based on the laboratory diagnosis under the supervision of quantified clinicians at Omkoi District Hospital, Omkoi District, Chiang Mai Province.

2.2. Sample collections

Ten milliters of whole blood samples were collected and delivered to the Clinical Service Center, Faculty of Associated Medical Sciences, Chiang Mai University for hematological and iron analysis. The teachers gave each participant child a cleaned labelled plastic containner and explained the procedure for collection of a single stool sample. Stool samples contaminated with water or urine were rejected. Stool samples were kept in ice-boxing during transportation to the parasitology labolatory, Department of Parasitology, Faculty of Medicine, Chiang Mai University and then analyzed immediately.

2.3. Intestinal parasite detection

Approximately 2 gm of individual fecal samples were stirred well in a 15-mL Falcon tube containing 10 mL of 10% formalin (Merck, Darmstadt, Germany) for fixation. Formalin-fixed samples were then analyzed for intestinal helminths and protozoa using formalin ether concentration method (World Health Organization, 1991; Garcia, 2016) with minor modifications. Briefly, the preserved fecal suspension was filtered through two layers of gauze into a centrifuge tube and the volume was adjusted to 10 mL with 10% formalin. Three mL of ether (Merck, Darmstadt, Germany) was added, sealed with a stopper and shook vigorously in an inverted position for 30 s. The tube was then centrifuged at 500*g* for 10 min. The plug of debris along with the formalin-ether solution was discarded by inverting the tube, leaving only the sediment, which was resuspended. The entire suspension was examined under microscope.

2.4. Hematological determination

Hematological parameters were measured using an automated blood counter SIEMEN ADVIA 2120i (Siemens Healthcare Diagnostics, Tarrytown, NY, USA). Classification of anemic and non-anemic children was performed according the reference values (World Health Organization, 2008). Anemic children were classified when hemoglobin (Hb) concentration is < 11.5 g/dL for children aged 5–11.9 years and < 12.0 g/dL for those 12–14.9 years old. Eosinophilia was defined as more than 500 cells/μL of an absolute eosinophil count, in the peripheral blood (Keohane et al., 2015).

Detection of α-thalassemia-1 was carried out by using molecular diagnostic method. Genomic DNA was extracted from whole blood sample using NucleoSpin® kit (Macherey-Nagel, KG., Duren, Germany) according to manufacturer's instructions. The molecular diagnosis of α-thalassemia-1 Southeast Asian (SEA) and Thai type deletions was performed using SYBR Green1 quantitative PCR with high resolution melting (HRM) analysis (Pornprasert et al., 2008). Detection of β-thalassemia and hemoglobinopathy including Hb E was performed using high performance liquid chromatography (HPLC; VARIANT II™, β-Thalassemia Short Program; Bio-Rad Laboratories, Hercules, CA, USA). The β-thalassemia trait, Hb E trait, β-thalassemia/Hb E or homozygous Hb E were classified when level of Hb A_2/E is 4–9.9%, 10–29.9%, 30–60% (with Hb F ≥ 15%) and > 65%, respectively (Pornprasert et al., 2010).

Detection of G-6-PD deficiency was analyzed using a fluorescent spot test (Beutler et al., 1979) with minor modifications. In brief, 10 μL of whole blood sample was added to 200 μL of G-6-PD screening reagent (Sigma-Aldrich, MO, USA) which was prepared immediately prior to use. The solution was then incubated at 37 °C in the dark for 10 min. An aliquot of the solution was spotted onto a Whatman filter paper, air dried and examined under UV light. Control normal and G-6-PD deficient blood samples were included in each assay.

2.5. Serum iron determination

Serum iron parameters, including serum iron (SI) total iron binding capacity (TIBC) and serum ferritin were measured using an automated chemistry analyzer. SI and TIBC, and serum ferritin were analyzed by using the Roche Cobas c501 and e601, respectively (Roche Diagnostics, Indianapolis, IN, USA). Transferrin saturation was calculated using the formula: (SI/TIBC) × 100. The iron deficiency (ID) was defined as transferrin saturation of less than 16% and/or serum ferritin level of less than 10 ng/mL (Keohane et al., 2015). IDA was defined as iron deficiency concurrent with anemia.