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Study of Association between Anemia and Worm Infestation among the Tea Estates Workers in Ilam District, Eastern Region of Nepal

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ABSTRACT

Introduction: Anemia constitutes a worldwide problem and at present is a major tropical disease. Iron deficiency anemia is reported from India, Africa, and South America. Objectives: To measure the prevalence of anemia and to find the association between anemia and worm infestation among the tea garden workers in Ilam district of Nepal. Materials and Methods: A Community based cross-sectional study was conducted among tea garden workers in Ilam district of Nepal. Out of 4 tea estates in Ilam District, 2 tea estates (Ilam Municipality and Kanyam) were selected randomly. Out of total 150 tea workers (30 in Ilam Municipality and 120 in Kanyam), 98 workers participated in the study. A semi-structured questionnaire was administered to the study subjects and Microscopic Examination of Stool was done. Hemoglobin level was determined by cyanmethemoglobin method. The Chi square test was used to find the association between anemia and worm infestation among the tea garden workers in Ilam district of Nepal. Results: Overall prevalence of anemia among the tea garden workers were found to be 43.9 percent. The prevalence of anemia was seen significantly higher in female (53.3%) than male (13%) ($P<0.05$). The anemia was higher among study population who was below School leaving certificate (SLC) (50%) than SLC & above SLC (14.3%) but the difference was not significant. The prevalence of anemia was seen higher among the study population infected with worm (61.1%) than worm negative (40%) but the difference was not significant. Mild anemia was seen significantly higher (54.5%) among worm positive in comparison to moderate anemia (45.5%) ($P<0.05$). The prevalence of anemia was seen significantly higher among the study population infected with hookworm (90%) than other worms including Ascaris lumbricoides, Trichuris trichuria and Hymenolepsis nana (38.6%) ($P<0.05$). Conclusions: Anemia and worm infestations remain important public health problem in the Nepal. These findings suggest that it is necessary to develop effective prevention and control strategies of worm infestation including health education and environmental hygiene.

INTRODUCTION

Soil-transmitted helminth (STH) infections are among the most common infections, primarily affecting the poorest sectors of the population. In 2010, an estimated 819 million people worldwide were infected with *Ascaris lumbricoides*, 464 million with *Trichuris trichura*, and 438 million with hookworm.[1]

Iron-deficiency anemia is the most prevalent nutritional deficiency worldwide.[2] More than 90% of affected individuals live in the developing world, where helminth infections are highly prevalent and the parasites are endemic.[2] Helminths are known to be significant contributors to the overall anemia burden in the developing world.[3] The negative impact that high-intensity helminth infections have on hemoglobin levels has been convincingly demonstrated through observational and interventional studies of many populations.[3,4] Intestinal parasitic infections, particularly from *Ascaris lumbricoides*, *Trichuris trichiura* and the two hookworm species Ancylostoma duodenal and Necator Americans are often associated with conditions such as malnutrition, vitamin A deficiency, diarrhoea and iron deficiency anaemia.[5] Hookworms* and Trichuris may cause anemia by consuming blood and causing plasma leakage.[6]

Non-mechanized, agricultural labor is the main source of family income in most developing countries, where lower labor productivity due to poor health has profound economic consequences. A tea plantation provides ideal conditions for studying labor productivity as work attendance and weight of the green leaves plucked by each worker are carefully recorded in order to calculate individual wage packets.[7] Therefore this study was designed to measure the prevalence of anemia and to find the association between anemia and worm infestation among the tea garden workers in Ilam district of Nepal.

METHODOLOGY

A Community based cross-sectional study was conducted from 13th December 2015 to 27th December 2015 in tea garden workers in Ilam district of Nepal. This was a two weeks study to fulfill epidemiological management carried out by students of MBBS 3rd year Batch 2013 of B. P. Koirala Institute of Health Sciences, Dharan, Nepal. This research was based on random selection of the study area Ilam District. Four tea estates under Nepal Tea Development Cooperation (NTDC) at Ilam District are Ilam Municipality, Kanyam, Soktim, and Chilimkot. Out of 4 tea estates of Ilam District, 2 tea estates (Ilam Municipality and

Kanyam) were selected randomly. Out of total 150 tea workers (30 in Ilam Municipality and 120 in Kanyam), 98 workers participated in the study.

Ethical clearance was taken by Institutional Review Committee of B P Koirala Institute of Health Sciences, Dharan, Nepal. Written permission was taken from each in charge of Nepal Tea Development Cooperation (NTDC) at Ilam Municipality, Kanyam, and participants. Tea garden workers of both sexes, aged 18 years and above, having working experience of minimum 6 months and those who gave written consent were included in the study.

A semi-structured questionnaire was administered to the study subjects and Microscopic Examination of Stool was done. In each visit, more than 15 workers were enrolled & the same number of plastic bottles was given for stool collection and collected next day morning. Side by side blood samples was taken for the estimation of their hemoglobin level. Microscopic examination of stool was done by preparing slide using Normal Saline and Lugol's Iodine to observe the ova of different intestinal helminthic parasites. First, we used low power lens and afterward the high power lens. Then we observed ova of different intestinal helminthic parasites.[8] Hemoglobin level was determined by cyanmethemoglobin method. When a measured quantity of blood (20 µl) was diluted in 5 ml of Drabkin's solution, the hemoglobin was converted to cyanmethemoglobin. The hemoglobin content was then determined by spectrophotometer (540 nm).[9] The confidentiality and privacy of the study were maintained; a name of the individuals or participating group was not disclosed after the study.

All interviewed questionnaires were indexed and kept on file. Data were entered in Microsoft Excel and converted into SPSS (Statistical Package for Social Science) 11.5 version for statistical analysis. The prevalence was calculated, Chi-square test was used to measure the association between anemia and worm infestations. The confidence level was set at 5% in which probability of occurrence by chance is significant if $P < 0.05$ with 95% Confidence Interval.

RESULTS

Table 1: Distribution of anemia among study population

Characteristics	Frequency	Percent
Anaemia		
Positive	43	43.9
Negative	55	56.1
Total	**98**	**100.0**
Category of anemia		
Mild anemia	37	37.8
Moderate anemia	6	6.1
Total	**43**	**43.9**

Table 1 shows the status of anemia among the Tea Estates workers of Ilam District. A total anemia was found to be 43.9 percent. Out of 43 anemia cases, mild anemia and moderate anemia was found to be 37.8% and 6.1% respectively.

Table 2: Association between sociodemographic characteristics with anemia

Characteristics	Anemia		Total	P-Value
	Yes	No		
Age				
18-40 years	22 (47.8)	24 (52.2)	46	0.604
41-60 years	19 (42.2)	26 (57.8)	45	
>60 years	2 (28.6)	5 (71.4)	7	
Gender				
Male	3 (13.0)	20 (87.0)	23	0.001
Female	40 (53.3)	35 (46.7)	75	
Religion				
Hindu	30 (41.1)	43 (58.9)	73	0.343
Others (Muslim, Buddhist, Christian)	13 (52.0)	12 (48.0)	25	
Ethnicity				
Brahmin/Chhetri	17 (47.2)	19 (52.8)	36	0.367
Janajati	16 (50.0)	16 (50.0)	32	
Dalit	10 (33.3)	20 (66.7)	30	
Education				
Illiterate	26 (44.1)	33 (55.9)	59	0.226
Below SLC	16 (50.0)	16 (50.0)	32	
SLC & above SLC	1 (14.3)	6 (85.7)	7	
Total	**43 (43.9)**	**55 (56.1)**	**98**	

SLC: School leaving certificate

The prevalence of anemia was seen significantly higher in female than male (P<0.05). The prevalence of anemia was seen higher among Janajati than other ethnic groups (P>0.05). The anemia was higher among study population who was below School leaving certificate (SLC) than SLC & above SLC but the difference was not significant (Table 2).

Table 3: Association between anemia and worm infestation

Characteristics	Worm		Total	P-Value
	Positive	Negative		
Anemia				
Yes	11 (61.1)	32 (40.0)	43 (43.9)	0.103
No	7 (38.9)	48 (60.0)	55 (56.1)	
Total	18 (18.4)	80 (81.6)	98 (100)	
Category of Anemia*				
Mild Anemia	6 (54.5)	31 (96.9)	37(86.0)	0.003
Moderate Anemia	5 (45.5)	1 (3.1)	6(14.0)	
Total	11 (25.6)	32 (74.4)	43 (100.0)	

**Chi-square with continuity correction*

The prevalence of anemia was seen higher among the study population infected with worm than worm negative but the difference was not significant. Regarding category of anemia, mild anemia was seen significantly higher among worm positive in comparison to moderate anemia (P<0.05) (Table 3).

Table 4: Association between anemia and worm infestation (hookworm and other worms)

Characteristics	Worm		Total	P-Value
	Hookworm	Other worms		
Anemia				
Yes	9 (90.0)	2 (25.0)	11 (61.1)	0.005
No	1 (10.0)	6 (75.0)	7 (38.9)	
Total	10 (55.6)	8 (44.4)	18 (100.0)	

The prevalence of anemia was seen significantly higher among the study population infected with hookworm than other worms including *Ascaris lumbricoides*, *Trichuris trichuriasis* and Hymenolepsis nana (P<0.05) (Table 4).

DISCUSSION

Anemia constitutes a worldwide problem and at present is a major tropical disease. Severe iron deficiency anemia is reported from India, Africa and South America. Though the aetiology of these anemia may be manifold, it has become obvious that chronic blood loss caused by certain parasitic and helminthic infections plays an important role in their causation.[10] The hookworm is particularly prevalent throughout much of sub-Saharan Africa as well as in South China, the Pacific and Southeast Asia. However, this picture may underestimate the true global distribution.[11] Jung [12] and Biagi[13] reported a microcytic, hypochromic anemia in Trichuris trichiurid infection. Layrisse et al measured the blood loss using 51Cr-tagged red cells in heavily infected people and reported a blood loss ranging from 0.8 to 8.6 mi/day and concluded that infections of over 800 parasites can induce anemia in people.[14]

A total anemia among the Tea Estates workers of Ilam District was found to be 43.9 percent. Out of which mild anemia and moderate anemia was found to be 37.8% and 6.1% respectively. A study conducted by Manna PK et al in Tea Garden Areas of Darjeeling and Jalpaiguri Districts of West Bengal, which showed the percentage of anemia, was 82.5%. Out of which 39.5% were suffering from mild anemia, 35% were suffering from moderate anemia and 6.7% were suffering from severe anemia which was higher than our study.[15] One study conducted by Pandit et al in Mumbai in 2005 showed that 77.7% of respondents were anemic out of which 25.9% respondents were moderate anemic and 5.2% were severely anemic. This study also focuses that the respondents are suffering more from moderate and severe anemia. [16] A study conducted in West Bengal by Das et al in 2005[17] and in other developing countries by Shah et al in 2002[18], and Agha et al in 1992[19] have shown a high prevalence of anemia, that is, between 25% to 88%. One study in Madhya Pradesh by Gawarika et al in 2006 indicated that overall prevalence of anemia was 96.5%.[20] Bulliyy et al in 2007 found 96.5% prevalence of anemia in three districts of Orissa of which 45.2%, 46.9%, and 4.4% had mild, moderate and severe anemia.[21]

The prevalence of anemia was higher among the workers who were below School leaving certificate (SLC) (50%) than SLC & above SLC (14.3%) but the difference was not significant. A study conducted by Banu H et al in Bangladesh in 2014 showed the anemia was also higher (37.2%) among the illiterate compared to the 15.3% whose education level was above secondary. Educational level of the respondents was also inversely related to the

prevalence of anemia ($p<0.01$). This implies that when educational level increased the prevalence of anemia tends to decreased.[22] Study by Gawarika et al in 2006 also showed that the education of respondents is significantly related to anemia. This study also focused on the significant relationship between educational level with anemia ($p<0.01$).[20] Educated people probably are more serious about the health status than the uneducated person is and that may be the cause of low prevalence of anemia in educated group.[15]

The level of education of the study population and parasitic infection was highly significant ($p<0.000$). The exposure (Illiterate) was positively associated with parasitic infection (OR=4.73). The risk of exposure was 2.96 times higher than non-exposure (literate) to form positive parasitic infection.[22] The educational level of the respondents was significantly associated with anemia ($P<0.001$). The exposure (illiterate) was positively associated with anemia (OR=1.68). The risk of exposure was 1.43 times higher than non-exposure (literate) to occur anemia.[22] Ahmed et al in 1993 reported higher hemoglobin level among the study population when the education level found increased.[23]

This study showed the prevalence of anemia was seen higher among the workers infected with worm (61.1%) than worm negative (40%) but the difference was not significant. Regarding category of anemia, mild anemia was seen significantly higher among worm positive (54.5%) in comparison to moderate anemia (45.5%) ($P<0.05$)**.** A study conducted by Banu H et al in Bangladesh in 2014, which showed that prevalence of intestinal parasite among anemic cases, was higher than on anemic cases in all study areas. It may be mentioned here in anemic cases, the highest rate of infection was found 55.3% in Kamrangirchar and the second highest rate 50.7% in Zinjira. Out of total 506 (32.2%) anemic cases in the study, 59.1% were mildly anemic, 33.2% were moderately and 7.5% were severely anemic.[22] Other studies reported that parasitic infestation is one of the causes of anaemia.[24, 25] Shah and Baig in 2005 reported that anemia significantly related with helminth infection.[26]

Other studies in Peru reporting the similar prevalence of anemia also demonstrate the high prevalence of STH infections.[27] While anemia was not associated with helminth infections in the study, the association with eosinophilia suggests a parasitic cause.[28] recently, much progress has been made in measuring quantitatively the relationship between the intensity of parasitic infection, anemia, and blood loss, and the major part of this discussion will, therefore, be directed towards defining the role played by the common helminthic infections in causing blood loss and iron-deficiency anemia.[10] The helminths associated with iron

deficiency anemia are those causing chronic blood loss from either the gastrointestinal tract or urinary system. These include hookworm infection (Necator Americans and A ancylostoma duodenal), whipworm infection (*Trichuris trichiura),* and schistosomiasis (*Schistosoma mansion, S. crematorium, and S. japonicum*).[10]

This study showed that prevalence of anemia was significantly higher among the workers infected with hookworm (90%) than other worms including *Ascaris lumbricoides*, *Trichuris trichuria* and *Hymenolepsis nana* (25%) ($P<0.05$). Perroncito[29], in 1880, first noted a relationship between hookworm infections and anemia, and Darling et al[30] in 1920 emphasized the direct relationship between the hookworm infection and anemia. Reports from China[31], Mexico[32], India[33], United States[34], Mauritius[35,] Egypt[36], and Venezuela[37] confirmed that with increasing loads of hookworm infection there occurred a concomitant decrease in hemoglobin concentration. Intestinal blood loss is the major clinical manifestation of human hookworm infection (for a review of studies, Roche and Layrisee (1966), Miller (1979), and Crompton and Stephenson (1990)).[38,39,40] Heavy hookworm infections or moderate infections in patients with underlying iron and protein nutritional deficiencies result in hookworm disease, the clinical entity that specifically refers to the resulting iron deficiency and microcytic, hypochromic anemia.[41] Roche et al[42] reported a blood loss of 0.03 ml/day for each Necator Americans worm, Tasker[43] reported a blood loss of 0.04-0.1 ml/worm per day, Gilles et al[44] approximately 0.05 ml/day, and Mahmood[45] 0.005-0.13 mi/worm per day.

The role of hookworm in causing anemia is well documented. Hookworms injure their human host by causing intestinal blood loss leading to iron deficiency and protein malnutrition.[46,47] The parasite induces blood loss directly through mechanical rupture of host capillaries and arterioles followed by the release of a battery of pharmacologically active polypeptides including anticoagulants, antiplatelet agents, and antioxidants.[48,49] Hookworms subsequently digest host hemoglobin by employing a carefully orchestrated cascade of hemoglobin aces that align the brush border membrane of the parasite's alimentary canal.[50,51] Although the threshold might be expected to be well established because of the accurate estimates of blood loss caused by each hookworm species[52], the precise value is actually community dependent because the onset of anemia is dependent on the iron status and reserves of the host[53]. This, in turn, depends on a number of factors including dietary iron intake and overall level of nutrition.

The present study has limitations that may prevent more robust conclusions from being drawn. Firstly, the small sample size and geographic area studied limited the power of the analysis and affected the generalizability of results. Secondly, we conducted single stool examination for detection of intestinal parasitic infections, which could have underestimated the prevalence, as optimal laboratory diagnosis of intestinal parasitic infections requires the examination of at least three stool specimens collected over several days.[54] Despite limitations, the current data add to the scarce literature about anemia in the Nepal and highlight the need for further research and comprehensive interventions to improve these health indicators.

CONCLUSION

The overall prevalence of anemia was seen high among the tea garden workers in Ilam district of Nepal. The main parasites causing blood loss in man and leading to direct iron deficiency anemia are the common worm infections. The prevalence of anemia was seen significantly higher among the workers infected with hookworm than other worms including Ascaris lumbricoides, Trichuris trichuria, and Hymenolepsis nana. Large-scale treatment programs are currently underway, supported by health education and integrated with the provision of improved water and sanitation. There are also efforts underway to develop novel antihelminthic drugs and anti-hookworm vaccines.

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