e ISSN 2277 - 3290
Print ISSN 2277 - 3282

Journal of Science

www.journalofscience.net

STUDY OF ASSOCIATION BETWEEN ANEMIA AND PROTOZOAN INFESTATION AMONG THE TEA ESTATES WORKERS IN ILAM DISTRICT, EASTERN REGION OF NEPAL

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ABSTRACT

Anemia is a public health concern in developing countries especially among vulnerable populations. The prevalence of anemia associated with intestinal protozoan infections among the Tea Estates workers in the rural communes of Nepal is largely unknown. Objectives to find out the association between anaemia and protozoan infestation among the tea garden workers in Ilam district of Nepal. 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. Semi-structured questionnaire was administered to the study subjects and Microscopic Examination of Stool was done. Haemoglobin level was determined by cyanmethemoglobin method. The Chi-square test was used to find the association between anaemia and protozoan infestation among the tea garden workers in Ilam district of Nepal. The prevalence of anaemia was seen significantly lower among study population infected with protozoa (18.2%) than protozoa negative (51.3%) ($P<0.05$). Regarding category of anemia, mild anaemia was seen higher (75%) among protozoa positive in comparison to moderate anemia (25%) but the difference was not significant. The prevalence of anaemia was seen higher among the tea garden workers infected with Entamoeba histolytica (20%) than Giardia lamblia (16.7%) but the difference was not significant ($P>0.05$). The prevalence of anaemia was seen lower among the tea garden workers infected with protozoa. Anaemia was seen higher among workers infected with Entamoeba histolytica than Giardia lamblia.

Keywords: Anaemia, Protozoan infestation, Tea Estates workers, Ilam, Nepal.

INTRODUCTION

The prevalence of anemia throughout the world is high, and anemia affects every segment of the population. The WHO estimates that mild to moderate cases are associated with reduced physical and mental capacity. Severe cases could result in morbidity and mortality in all population groups. Anemia is a physical condition that is defined as having a hemeglobulin (Hb) level below a threshold value, which could be caused by several factors. Nine out of ten people who suffer from anemia live in developing countries. In rural areas the prevalence is often higher as nutritional deficiencies and parasitic infection often prevail within the same individuals [1]. Iron- deficiency anemia is a major public health problem all over the world, particularly in the developing countries [2]. Malnutrition in addition to infection with protozoan or helminth parasites alone or the combination of both is generally regarded as the major underlying factors. It was shown that severe infection, with Giardia intestinalis is associated with iron-deficiency anemia [3]. However several studies have recorded that the most common mineral deficiency in nutritional anemia is iron deficiency [4,5].

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Iron deficiency anemia is considerably more prevalent in developing countries than in the industrialized world [6]. There is some evidence that a number of protozoan parasites can interfere with the absorption of some nutrients particularly if the worm burden is high [7]. Contrary to this, some investigators have reported no significant hematological alterations due to single protozoan infection, but only in case of major structural and functional abnormalities of the small intestine [8,9]. Epidemiological studies dealing with iron-deficiency anemia caused by protozoan parasites are few. In Nepal, few studies made so far mainly deal with helminthes parasites [10]. Thus, evidently there is dearth in information about anemia in relation to protozoan infection. Therefore, the present study is undertaken to find out the association between anaemia and protozoan 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 incharge 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. Semi-structured questionnaire was administered to the study subjects and Microscopic Examination of Stool was done. In each visit more than 15 workers was enrolled & same number of plastic bottles was given for stool collection and collected next day morning. Side by side blood samples were 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 protozoan parasites. First we used low power lens and afterwards the high power lens. Then we observed ova of different intestinal protozoan parasites.[11] Haemoglobin level was determined by cyanmethemoglobin method. When a measured quantity of blood (20 µl) was diluted in 5 ml of Drabkin's solution, the haemoglobin was converted to cyanmethemoglobin. The haemoglobin content was then determined by spectrophotometer (540 nm) [12]. The confidentiality and privacy of the study was maintained; name of the individuals or participating group was not disclose after the study.

All interviewed questionnaires were indexed and kept on file. Data was entered in Microsoft Excel and converted into SPSS (Statistical Package for Social Science) 11.5 version for statistical analysis. Chi-square test was used to measure the association between anaemia and protozoan 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.

The prevalence of anaemia was seen significantly lower among the tea garden workers infected with protozoa than protozoa negative ($P<0.05$). Regarding category of anemia, mild anaemia was seen higher among protozoa positive in comparison to moderate anemia but the difference was not significant (Table 1).

The prevalence of anaemia was seen higher among the tea garden workers infected with Entamoeba histolytica than Giardia lamblia but the difference was not significant ($P>0.05$) (Table 2).

RESULTS

Table 1. Association between anaemia and protozoa infestation

Characteristics	Protozoa		Total	P-Value
	Positive	Negative		
Anaemia				
Yes	4 (18.2)	39 (51.3)	43 (43.9)	
No	18 (81.8)	37 (48.7)	55 (56.1)	0.006
Total	22 (22.4)	76 (77.6)	98 (100.0)	
Category of Anemia*				
Mild Anemia	3 (75.0)	34 (87.2)	37 (86.0)	
Moderate Anemia	1 (25.0)	5 (12.8)	6 (14.0)	0.503
Total	4 (9.3)	39 (90.7)	43 (100.0)	

Table 2. Association between anaemia and different types of protozoan infestation

Characteristics	Protozoa			P-value
	Entamoeba histolytica	Giardia lamblia	Total	
Anaemia				
Yes	2 (20.0)	2 (16.7)	4 (18.2)	0.840
No	8 (80.0)	10 (83.3)	18 (81.8)	
Total	10 (100.0)	12 (100.0)	22 (100.0)	

DISCUSSION

Intestinal parasitic infection (IPI's) caused by pathogenic helminthes and protozoan species are endemic throughout the World. Intestinal parasitic infections (IPI) constitute a global health burden causing clinical morbidity in 450 million people in developing countries [13]. The major IPI's of global public health concern are the protozoan species Entamoeba histolytica and Giardia intestinalis and the soil transmitted helminthes A. lumbricoides, T. trichiura , and Hookworm [14]. Amoebiasis, Ascariasis, Hookworm infection and Trichuriasis are among the ten most common infections in the world [15]. The incidence and prevalence of these parasitic pathogens varies both between and within countries [16]. All these parasites had also been reported from Terai area Sarlahi District (Nauvilsky et al. 1998) [17], in hilly area i.e. Dhankuta District (Shah) of Nepal. The occurrence of parasitic infection at high rates is an indicative of faecal pollution of soil, domestic water supply around homes due to poor sanitation, ignorance of the mode of transmission of these parasites and improper utilization of latrine and poor personal hygiene among the study population [18].

This study showed Etaboeba histolytica and Giardia lamblia are common protozoan infections among the protozoan parasites. In Bangladesh, among the protozoan parasites, Etaboeba histolytica and Giardia lamblia are common. The protozoan infection creates different public health problems among the hosts directly or indirectly and can cause nutritional impairment, retard physical and mental development of the people [19,20].

Anemia is still a major public health problem in many developing countries. The World Health Organization (WHO) Global Database on Anemia for 1993-2005 showed that 25% or 1.62 billion people globally suffer from anemia with the highest number of 315.4 million in South-East Asia region [21]. Anaemia, one of the Worlds nineteenth leading risk factor for death and disability adjusted life year (DALY) is an indicator of poor nutrition and health with major consequences for health, social and economic development of a population [22]. Anaemia is recognized to be public health problem and both nutritional (such as iron and other mineral and vitamin deficiencies) and non-nutritional (such as infection, infestation and haemoglobinopathies) factors contribute to the onset of anaemia and iron deficiencies. [23,24]. A total anemia among the Tea Estates workers of Ilam District was found to be 43.9 percent (Table 1). In hospital based study done by Bonevik et al (2000) [25] showed prevalence of anemia 62.2% in Kathmandu, Nepal while Adak and Nazri in 2006 [26] showed 54.6% in another study conducted in Birgunj, Nepal. Similarly high prevalence (50% - 60%) (Ulstein et al. 1988) [27]. of anaemia were noted in various community based studies particularly important studied carried out by Shah and Gupta (2002) [28]. showed that prevalence of anaemia in Dharan, a town in eastern region of the country was 68.8%. All these data showed higher prevalence of anemia than our study.

Regarding category of anemia, mild anemia was seen higher (86%) in comparison to moderate anemia (14%) (Table 1). A study conducted by Toteja GS et al in 16 districts of India which showed Dibrugarh is one of the 16 Districts where anemia prevalence as 93.3% with 35.5% mildly anaemic, 49.9% moderately anaemic and 8.8% severely anaemic [29].

A study in Nepal found high prevalence of parasitic infestations where the associated morbidities like anemia and reduced resistance due to other nutritional disorders made the condition worse and parasitic infestation further aggravated anaemia. [30]. The study done by Shah and Gupta in the eastern region of the country also showed a 68.8% prevalence of anaemia among study population. A study done in Nepal by Curtale et al showed a significant relationship of anemia with parasitic infestation [31]. Other studies also found a strong association between anaemia and parasitic infestations [32,33].

Infections were higher (39.5%) amongst anaemic cases than the non anaemic cases Parasitic (30.6%). Anaemia was significantly associated ($P<0.001$) with parasite infection. Odds Ratio (OR=1.48) showed that exposure (anaemic cases) was positively associated with parasitic infection. Relative Risk (RR=1.29) showed that risk of exposure was 1.29 times higher than non-exposure (not anaemic) to form positive parasitic infection.[34] Other studies reported that parasitic infestation is one of the causes of anaemia (Banu et al 2011, Banu and Khanum 2013) [35,36]. The study had shown (55%) of study population were anaemic patient, among those anaemic 36.3% had parasitic infection, while 45% of study population were non-anaemic with 20% among them with parasitic infection. The prevalence rate shows the association of anaemia with intestinal parasite was statistically significant ($P<0.05$).

The study population having multiple infections showed the lowest mean Hb levels of 9.81} 0.84 gm/dl (moderate anemia) while the mean Hb levels of study population infected with single parasite was 10.4} 1.80 gm/dl (mild anaemia) [37]. The present study showed the prevalence of anaemia was seen significantly lower (18.2%) among the tea garden workers infected with protozoa than protozoa negative (51.3%) ($P<0.05$) (Table 1). The prevalence of anaemia was seen higher among the tea garden workers infected with Entamoeba histolytica (20%) than Giardia lamblia (16.7%) but the difference was not significant ($P>0.05$) (Table 2). But a study conducted by Alzain BF et al in Udaipur City, India showed that the study population suffering from protozoan parasitic infection have significant hematological disturbance, as manifested by the lower hemoglobin level indicating the presence of anemia, compared to control subjects [38]. However, several investigators have reported lower levels of hemoglobin among the study population [39,40]. They showed that the study population infected with pathogenic intestinal parasites in different age groups and on the whole have an apparent higher prevalence of anemia than those of non-infected. An association between the high prevalence of E. histolytica and G. intestinal with anemia was observed in the study population.

It was reported that patients with giardiasis and the study population harboring mixed protozoan infection as well as double infection (protozoan + helminthes) showed comparatively low hemoglobin level than those having single infection [41,42]. The anemia in the study could be attributed to double and mixed parasitic infections. The prevalence of anemia was found to be 70 % in Punjabi and 62.8 % in India [43]. The study indicated that following anti-protozoan treatment significant improvement was reported in the hemoglobin levels of most of the study population with single and mixed protozoan infection. The health status of the study population in Udaipur, India was very poor as illustrated by 65% were having anemia. However, the low hemoglobin levels which were found in both the infected and non-infected the study population probably indicate nutritional anemia rather than anemia of parasitic origin. Similarly, it was reported 62% of the study population in Zanzibar have anaemia due to iron deficiency [44].

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.[45] 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 prevalence of anaemia was seen high but significantly lower among the tea garden workers infected with protozoa than noninfected. Anaemia was seen higher among the workers infected with Entamoeba histolytica than Giardia lamblia. Therefore, High anaemia prevalence requires urgent attention to avoid preventable morbidities. Implementation of different intervention in an integrated manner was found effective in reducing the burden of anaemia and associated factor.

ACKNOWLEDGMENT

We would like to thank to School of Public Health and Community Medicine for approval of our research work. We would like to acknowledge the 5th semester students of MBBS Batch of 2013 who helped us during the study period and participants of the tea garden workers in Ilam district of Nepal for their kind co-operation.

CONFLICT OF INTEREST: No conflict of interest

FUNDING: None

REFERENCES

1. Hoa NV, Thang VV. Anemia, meat consumption and hookworm infection in women of reproductive age in the Nam Dong mountainous District, Thua Thien Hue Province. *Journal of Science*, 61, 2010, 185-199.
2. World Health Organization, control nutritional anemia with especial reference to iron deficiency. Technical Report Series, 580 Report of IAEA/ USAID/ WHO, Joint meeting, 1975.
3. Poly JR, Rosenfield S. Malabsorption in Gairdiasis presences of luminal barrier (mucoid pseudomembrane) a scanning and transmission electron microscopic study. *Journal Pediatric Gastroenterology and Nutrition*, 1, 1982, 63.
4. Palti H, Presence B, Alder B. Does anemia in infancy achievement on developing and intelligence tests. *Human Biology*, 55, 1982, 183-194.
5. Hercberg S, Galan P, Assami M, Assami A. Evaluation of the frequency of anemia and iron deficiency anemia in a group of Algeria menstruating women by mixed distribution processes in the contribution of total deficiency and inflammatory processes in the determination of anemia. *International Journal of Epidemiology*, 17, 1988, 136-141.
6. De Maeyer EM. Preventing and controlling iron deficiency anemia through primary health care, WHO, Geneva, 1984.

7. De Vizia B, Poggi V, Vajro P, Cucchiara S, Acampora A. Iron malabsorption in Gairdiasis. *Journal of Pediatric*, 107, 1985, 75.
8. Maik SR, Mohant D, Rau NR, Vinagak VK. Hemoglobin profile in patients with G. lumblia infection. *Annals of Tropical Medicine and Parasitology*, 76 (1), 1982, 83-88.
9. Tewari SG, Tondon BN. Function and histological changes of small bowel in patient with G. lumblia infection. *Indian Journal of Medical Research*, 62, 1974, 689-695.
10. Shah BK, Baig LA. Association of anemia with parasitic infestation in pregnant Nepalese women: results from a hospital based study done in Eastern Nepal. *J Ayub Med Coll Abbottabad*, 17 (1), 2005, 5-9.
11. Godkar PB, Godkar DP. Microscopic examination of stool specimen. Text Book of Medical Laboratory Technology. 2nd ed. Mumbai: Bhalani Publishing House; 2003, P. 937-52.
12. Godkar PB, Godkar DP. Determination of haemoglobin level by cyanmethemoglobin method. Text Book of Medical Laboratory Technology. 2nd ed. Mumbai: Bhalani Publishing House; 2003, P. 727-729.
13. Quihui L, Valencia ME, Crompton DW, Phillips S, Hagan P, Morales G. Role of the employment status and education of mothers in the preva lence of intestinal parasitic infections in Mexican rural school children. *BMC Public Health*, 6, 2006, 225.
14. World Health Organization. Nutrition in South-East Asia. New Delhi, WHO. Regional Office for South-East Asia: New Delhi, 2000.
15. World Health Organization. Public health significance of intestinal parasitic infections. *Bulletin of the World Health Organization*, 65 (5), 1987, 575-588.
16. Mata L. Sociocultural Factors in the Control and Prevention of Parasitic Diseases. *Reviews of Infectious Diseases*, 4, 1982, 871-79.
17. Nauvilsky RC, Dreffuss ML, Shrestha J, Khatry SK, Stoltzfus RJ, Albonico M. Ancylostoma duodenale is responsible for hookworm infections among pregnant women in the rural plains of Nepal. *Journal parasitology*, 84, 1998, 347-651.
18. Rodriguez-Morales AJ, Barbella RA, Case C, Arria M, Ravelo M, Perez H et al. Intestinal Parasitic Infections Among Pregnant Women in Venezuela. *Infectious Diseases in Obstetrics and Gynecology*, 2006, 1-5.
19. Banu H, D'silva J, Islam N. Epidemiological factors and pinworm infection in children. *Bangladesh J Zool*, 31 (2), 2003, 243-246.
20. Khanum H, Ahmed S, Uddin MH, Rahman ABMM, Dey RR, Farhana M. Prevalence of intestinal parasites and anaemia among the slum male children in Dhaka city. *Dhaka Univ J Biol Sci*, 17 (2), 2008, 137-145.
21. Benoist BD, McLean E, Egll I, Cogswell M. Worldwide prevalence of anaemia 1993-2005: WHO global database on anemia, 2008.
22. World Health Organization. Global health risks: mortality and burden of disease attributable to selected major risks. 2009: pg-10.www.who. Int/healthinfo/global_ burden_ disease/ GlobalHealthRisks_report/pdf.
23. Agarwal DK, Upadhyay SK, Tripathi AM, Agarwal KN. Nutritional status, physical work capacity and mental function in school children. Scientific report 6, Nutrition Foundation of India. 1987 (http://www.unu.edu/ Unupress/food2/ UID09E /uid0 9e 1b. htm. (Accessed on 24th May 2016)
24. Raina N, Gupta A, Sharma M. Operational Study on Nutritional Anemia in Pregnant Women, Lactating Women and Adolescent Girls in a Rural Community in India. In Improving the Quality of Iron Supplementation Programs. Mother Care Project/ USAID/ John Snow, Inc. 1997, http: //www.fhi.org. (Accessed on 24th May 2016).
25. Bondevik GT, Ulstein M, Lie RT, Rana G, Kvale G. The prevalence of anemia in pregnant Nepali women- a study in Kathmandu. *Acta Obstetriciat Gynecologica Scandinavica*, 79 (5), 2000, 341-349.
26. Adak M, Nazri S. Prevalence of anemia among young girls and pregnant women of Birgunj, Nepal. *Journal of Institute of Medicine*, 28 (2), 2006, 1993-2979.
27. Ulstein M, Rana G, Yangzom K, Gurung R, Karki A, Gurung G. Some fetal and pregnancy parameters in Nepal. *Acta Obstetriciat Gynecologica Scandinavica*, 67, 1988, 47-52.
28. Shah BK, Gupta P. Weekly versus daily iron and folic acid supplementation in adolescent Nepalese girls. *Archives of Adolescent Pediatric Medicine*, 156 (2), 2002, 131-5.
29. Toteja GS, Padam S, Dhillon BS. Prevalence of anaemia amongst pregnant women and adolescent girls in 16 districts of India. *Food Nutr Bull*, 27, 2006, 311- 5.
30. Grover JK, Vats V, Uppal G, Yadav S. Anti-helminthics: a review. *Trop Gastroenterol*, 22 (4), 2001, 180-9.
31. Curtale F, Tilden R, Muhilal, Vaidya Y, Pokhrel RP, Guerra R. Intestinal helminthes and risk of anaemia among Nepalese children. *Panminerva Med*, 35 (3), 1993, 159-66.
32. Murthy GL, Sahay RK, Srinivasan VR, Upadhaya AC, Shantaram V, Gayatri K. Clinical profile of falciparum malaria in a tertiary care hospital. *J Indian Med Assoc*, 98 (4), 2000, 160-169.
33. Singh N, Shukla MM, Sharma VP. Epidemiology of malaria in pregnancy in central India. Bull World Health Organ, 77 (7), 1999, 567-72.

34. Banu H, Khanum H, Hossain MA. Relationships between anaemia and parasitic infections in adolescent girls of Bangladesh. *Bangladesh J Zool*, 42 (1), 2014, 91-103.
35. Banu H, Khanum H, Hossain MA. Parasitic infestation among the adolescent girls of Bangladesh. Advance in Parasitology: A novel approach towards a disease free world. University of Kalyani, Kolkata, India, 2011, pp 91-97.
36. Banu H, Khanum H. Intestinal parasitosis with anaemia and nutritional status: adolescent girls of Bangladesh. LAMBERT Academic Publishing (LAP) GmbH & Co. KG Heinrich- Böcking-Str. 6-8 66121, Saarbrücken, Germany, 2013, Pp. 308.
37. Chaudhary M, Maharjan M. Association of Anaemia with Parasitic Infection in Pregnant Women Attending Antenatal Clinic at Koshi Zonal Hospital. *Nepalese Journal of Zoology*, 2 (1), 2014; 1-7.
38. Alzain BF, Sharma PN. Hemoglobin Levels and Protozoan Parasitic Infection in School Children of Udaipur City (India). Journal of Al Azhar University-Gaza (Natural Sciences), 8, 2006, 35-40.
39. Ghaffauri HM, AL-fares AM, Islam SI, Ahmed AO, Jan MY. Hematological reference values assessed from birth to adolescence in Sand subjects in the area of Jeddah. *Sandi Medical Journal*, 8 (6), 1987, 575-582.
40. Khan MU, Amir SE, Eid OM, Aggarwal S. Prevalence of intestinal parasite among patients in Abha region. *Annual of Sandi Medicine*, 9, 1989, 471-474.
41. Tandon BN, Tandon RK, Katpathy BK. Hemoglobin values in case of Giardiasis. *Cut*, 18, 1977, 1176-1181.
42. Hartong WA, Courly WK, Arvantakis G. Giardiasis: Clinical spectrum and functional-structural abnormalities of the small intestinal muse. *Gastroenterology*, 77, 1979, 61-69.
43. Uberol IS, De Sweemer C, Taylor CE. A study of anemia among rural Punjabi children. *Indian Journal of Medicine Research*, 60, 1972, 793-799.
44. Stoltzfus RJ, Chwaya HM, Telsch J, Schulze KJ, Albonico M, Savioli L. Epidemiology of iron deficiency anemia in Zanzibar school children. *American Journal of Clinical Nutrition*, 65, 1997, 153-159.
45. Rashid MK, Joshi M, Joshi HS, Fatemi K. Prevalence of intestinal parasites among school going children in Bareilly District. *Natl J Integr Res Med*, 2, 2011, 35‑7.