

# Intestinal Parasitic Infections in Beta-thalassemia Major and Aplastic Anemia in a Tertiary Care Centre in Western India

Rajarshi Gupta<sup>1</sup>, Avani Koticha<sup>2</sup>, Preeti Mehta<sup>3</sup>

## ABSTRACT

**Introduction:** Beta-thalassemia major, one of the commonest hemoglobinopathies is not associated with any primary immune defect. But secondary immunological dysfunction does occur. Aplastic anemia is a rare hematologic disorder observed more frequently in Asian countries. Pancytopenia in aplastic anemia causes immunosuppression. Intestinal parasitic infections are prevalent in India. Hence, it is evident that such infections would affect patients with immune dysfunction and immunosuppression. However a thorough literature search revealed a lack of studies in this regard

**Material and Methods:** After obtaining Institutional Ethics Committee approval an observational, cross-sectional study was conducted at a tertiary care teaching hospital. 42 patients of aplastic anemia, 41 patients of beta-thalassemia major and 42 Hemophilia patients as controls were recruited after obtaining written informed consent. Stool examination was performed with respect to gross appearance and microscopy (saline and iodine mounts, formol ether sedimentation, modified acid fast staining and trichrome staining). Data was analyzed. *P* value of < 0.05 was considered significant.

**Results:** No significant difference in prevalence of intestinal parasitic infections in beta-thalassemia major patients or aplastic anemia patients was observed as compared to hemophilia patients (controls)

**Conclusion:** The various immune defects in beta-thalassemia major mentioned in literature do not seem to produce any profound immunosuppression. Cyclosporine in acquired aplastic anemia actually improved immune status. Thus patients were not particularly predisposed to intestinal parasitic infections. This study could not include large enough number of patients for extrapolation to larger population. Development of a time bound protocol for screening of intestinal parasites requires extensive studies on this population.

**Keywords:** Intestinal, parasites, aplastic, anemia, thalassemia, hemophilia, india, immunosuppression

## INTRODUCTION

Beta-thalassemia major is one of the commonest hematologic disorders encountered in India. Its prevalence varies from state to state but it is estimated to be 0.28 to 0.39 per 1000 live births in Western India.<sup>1</sup>

Beta-thalassemia major, a hemoglobinopathy though not associated with any primary defect in immune system,<sup>2</sup> yet secondary immunological dysfunction occurs as a consequence of iron overload and phagocytosis of RBCs in the spleen by macrophages, thereby causing a chronic immune stimulation.<sup>3-5</sup> It manifests as decreased CD4/CD8 ratio and diminished function of neutrophils, macrophages and natural killer (NK) cells. High immunoglobulin levels with poor differentiation has also been observed.<sup>3-5</sup> This might predispose the patient to various kinds of infections.

Aplastic anemia on the other hand is a rare hematologic disorder observed more frequently in Asian countries than in the West. It is estimated that 20-40% of pancytopenic patients in referral centres are aplastic anemia cases.<sup>6</sup>

As a tertiary care referral centre this hospital encounters frequent cases of aplastic anemia. Pancytopenia in aplastic anemia causes immunosuppression and predisposes the patient to infections.<sup>7</sup> Intestinal parasitic infections are prevalent in India.<sup>8</sup> Hence, it is quite evident that these infections would affect patients with immune dysfunction and immunosuppression. A thorough literature search revealed a lack of studies regarding intestinal parasitic infections in beta thalassemia and aplastic anemia in tropical countries possibly because beta-thalassemia is not considered to be a disease with immunologic defects and aplastic anemia is relatively rare. Majority of studies deal with transfusion related infections in beta-thalassemia major and aplastic anemia and bacteria, viral and fungal infections in aplastic anemia.<sup>3,9-11</sup>

Lack of protocol regarding frequency of screening of beta-thalassemia and aplastic anemia patients for intestinal parasitic infections, risk of serious and disseminated parasitic infections and diagnostic dilemma with hookworm infection in already anaemic patients prompted us to initiate a study to determine the prevalence of intestinal parasitic infections in beta-thalassemia major and aplastic anemia patients so that recommendations regarding necessity of regular screening for such infections could be put forward.

## MATERIAL AND METHODS

The study was initiated after obtaining Institutional Ethics Committee approval. It was observational and cross-sectional in nature and was conducted at a tertiary care teaching hospital for a duration of one year (January 2015 to December 2015)

On thorough literature search no similar study on patient population in tropical countries was found to enable sample size calculation. Hence, all patients fulfilling the inclusion criteria and consenting to the study during the said one year duration were included in the study.

42 patients of aplastic anemia and 41 patients of beta-thalassemia major admitted in the wards under Department of Hematology were recruited after obtaining written informed consent. For

<sup>1</sup>Speciality Medical Officer, <sup>2</sup>Professor (Addl.), <sup>3</sup>Professor and HOD, Department of Microbiology, Seth G.S. Medical College and K.E.M. Hospital, Parel, Mumbai, India

**Corresponding author:** Rajarshi Gupta, Department of Microbiology, Multi-storeyed Building (MSB), 5th Floor, Seth G.S. Medical College and K.E.M. Hospital, Parel, Mumbai -400012, India

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minors, written informed consent of the legal guardian was obtained. Patients of all ages and genders were recruited irrespective of symptoms. Patients on anti-parasitic medication or who had taken anti-parasitic medications in the previous two weeks, critical and unconscious patients and patients with transfusion related infections were excluded from the study. 42 Hemophilia patients admitted in the same ward during the same period were recruited as controls after obtaining written informed consent. This had been done to minimize confounding factors in the study. For minors, written informed consent of the legal guardian was obtained. Hemophilia is a bleeding disorder and is not known to cause any significant immune dysfunction. Hence, this was chosen as an appropriate control group for the study. Controls of all ages and genders were recruited irrespective of symptoms.

Hemophilia patients on anti-parasitic medication or who had taken anti-parasitic medications in the previous two weeks, critical and unconscious patients, patients with transfusion related infections and patients on immunosuppressants were excluded from control group.

Clinical history was obtained regarding the duration of illness, type of treatment administered and other symptoms suggestive of intestinal parasitic infections, defecation habits, place of residence (rural or urban) along with habit of barefoot walking outdoors.

Stool specimens were requested on three non-consecutive days, within a period of one week. Patients and/or their guardians were instructed to collect stool specimens in sterile wide mouthed screw capped plastic containers provided to them. They were asked to submit the specimen immediately to Parasitology Division, Department of Microbiology of the same hospital.

No stool preservatives were used. The specimens were processed as follows immediately on receipt.

**Gross examination** - Colour, Consistency, presence of blood, presence of mucus, worms, worm segments.

**Microscopic examination** - Direct wet mount (saline and iodine mounts), Flotation in saturated salt solution, Formol ether sedimentation technique, Trichrome staining, Modified kinyoun's acid fast staining

**Chemical examination** - Test for occult blood

After examination of the stool specimen, the report was dispatched to the respective wards and any finding was informed to the concerned clinician. For patients in whom parasites were detected, three stool specimens on non-consecutive days were requested after completion of treatment.

## STATISTICAL ANALYSIS

Data was analyzed using Chi square test and Mc Nemar test as required.  $P$  value of  $< 0.05$  was considered significant.

## RESULTS

Among patients of beta-thalassemia major 21.9% harboured intestinal parasites while 26.2% patients of aplastic anemia harboured intestinal parasites. Among the control group 14.3% patients of hemophilia harboured intestinal parasites. But there was no significant difference in prevalence of intestinal parasitic infections in beta-thalassemia major patients ( $P$  value = 0.36) or aplastic anemia patients ( $P$  value = 0.17) as compared to hemophilia patients (controls). Table-1 shows prevalence of intestinal parasites in patients with beta-thalassemia major. *Giardia lamblia* was the predominant intestinal protozoan detected followed by *Blastocystis hominis* and *Entamoeba histolytica/dispar* respectively. Among helminthic infections 1 case of *Strongyloides stercoralis* infection, 2 cases of *Ascaris lumbricoides* infestation and 3 cases of *Ancylostoma duodenale* infestation were detected. All patients with *Ancylostoma duodenale* infestation tested positive for occult blood in stool. Table-2 shows details of intestinal parasites detected in patients with beta-thalassemia major, aplastic anemia and hemophilia

## DISCUSSION

In this study observed prevalence of intestinal parasites in beta-thalassemia major, aplastic anemia and hemophilia were 21.9%, 26.2% and 14.3% respectively. But no significant difference was observed between any of the groups (Table-1). With respect to beta thalassemia major it might be said that this disorder does not produce any consistent immune defects. Immune defects are mostly secondary to the disease and not a primary manifestation.<sup>12</sup>

The various immune defects in beta-thalassemia major mentioned in literature do not seem to produce any profound immunosuppression to significantly increase the prevalence of

Condition	Total number of patients with intestinal parasites	Total number of patients without intestinal parasites	Total number of patients
Beta-thalassemia major	9(21.9%)	32(78.1%)	41
Aplastic anemia	11(26.2%)	31(73.8%)	42
Hemophilia	6(14.3%)	36(85.7%)	42
Total	26	99	125

**Table-1:** Prevalence of intestinal parasites in patients with beta-thalassemia major (n=41) aplastic anemia (n=42) and hemophilia (n=42)

Condition	Types of parasites detected with their numbers						Total number of patients with intestinal parasites
	Eh*	GI*	Bh*	AI*	Ad*	Ss*	
Beta-thalassemia major	2	3	3	1	0	0	9
Aplastic anemia	2	3	3	1	2 <sup>#</sup>	0	11
Hemophilia	1	2	1	0	1 <sup>#</sup>	1	6
Total	5	8	7	2	3	1	26

\* Eh - *Entamoeba histolytica/dispar*, GI - *Giardia lamblia*, Bh - *Blastocystis hominis*, AI - *Ascaris lumbricoides*, Ad - *Ancylostoma duodenale*, Ss - *Strongyloides stercoralis*; <sup>#</sup>All patients with *Ancylostoma duodenale* tested positive for occult blood in stool

**Table-2:** Details of intestinal parasites detected in patients with beta-thalassemia major, aplastic anemia and hemophilia

intestinal parasitic infections.<sup>13-15</sup>

With respect to aplastic anemia, all cases included in the study were patients of acquired aplastic anemia. Acquired aplastic anemia is primarily mediated by autoimmunity towards hematopoietic cells which causes pancytopenia and immunosuppression.<sup>7</sup>

These patients are maintained on cyclosporine till hematopoietic stem cell transplant can be performed. Cyclosporine is an immunosuppressive agent but in aplastic anemia it paradoxically improves immune status by dampening autoimmunity.<sup>16</sup>

All the aplastic anemia patients in our study were being maintained on cyclosporine which might have contributed to the patients having a favourable immune status.

Intestinal protozoa predominated the list of detected parasites (Table-2). No opportunistic coccidian parasites were detected in the study. There was one instance of *Strongyloides stercoralis* infection detected in a hemophilia patient. Despite best attempts no history of possible immunosuppression could be identified. However it was found that the patient was resident of a village and barefoot walking was a common practice. Two cases of *Ascaris lumbricoides* infection and three cases of *Ancylostoma duodenale* infection were detected. All five of these patients were residents of suburban rural areas. All three cases of *Ancylostoma duodenale* infection tested positive for occult blood in stool. Test for occult blood in stool is essential in these patients since detection of anemia provides no additional information in these already anaemic patients.

Post treatment, no intestinal parasites were detected in any patient.

#### Limitations of the study

The study could not include a large enough number of patients for the results to be extrapolated to a larger population. Sub group analysis of age, gender, symptomatic and asymptomatic patients could not be done as the sample size for subgroups were too small (< 30) for making any statistical conclusions.

Though less number of patients were included in the study yet the findings indicate requirement of regular screening for intestinal parasites in these cases. The development of a proper time bound protocol requires more extensive studies on this population. This would enable to reduce morbidity and mortality associated with these infections.

#### CONCLUSION

This study aims to serve as a preliminary one addressing the need for regular screening for intestinal parasites in patients with beta-thalassemia major and aplastic anemia. Though significant differences between various groups were not found in our study yet literature suggests immune dysfunction in beta-thalassemia major and aplastic anemia. More extensive studies could enable the development of a proper time bound protocol for screening of intestinal parasites in such patients.

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