

ORIGINAL ARTICLE

HAEMOGLOBIN A₂ LEVEL – A COMPARATIVE STUDY BETWEEN PATIENTS WITH MALARIA AND HEALTHY INDIVIDUALS

B M Jha¹, Arpita Nishal², Jitendra Patel³, Shailesh Lavanya⁴, Ritesh Shah⁴, Reena Dudhat⁵

¹Additional Professor, Department of Pathology & I/C Nodal Officer, Sickle Cell Laboratory, Sickle Cell Anemia Control Programme, ²Associate Professor, ⁴Senior Resident, ⁵Resident, Department of Pathology, ³Assistant Professor, Department of Immunohematology & Blood Transfusion, Government Medical College, Surat

Correspondence:

Dr Jitendra Patel
Assistant Professor,
IHTBT Department, Government Medical College,
Majura Gate, Surat – 395001. Gujarat, India.
E-mail: onlyg2@gmail.com, Phone: 91-9898585804

ABSTRACT

Background: Since many surveys for the prevalence of β thalassemia have been made in area with high prevalence of malaria, it is important to find out whether malaria can modify Hb A₂ levels. In present study, analysis of Hb A₂ level of patients with malaria and thalassemia has been done and has been compared with control group to conclude that whether Hb A₂ level was affected or not.

Methodology: Total 99 samples of malaria patients, 111 samples of β thalassemia trait patients & 105 samples of healthy individuals were tested. The p value of < 0.05 was used to consider difference as significant & to reject null hypothesis. The t test (two tailed distribution, two samples unequal variance) was used as hypothesis test.

Results: Hb A₂ level in malaria group ranged from 1.9 – 4.0 % (n = 89, mean 3.09 % & SD 0.32 %); those of the β thalassemia trait group ranged from 4.0 – 7.3 % (n = 111, mean 5.37 % & SD 0.74 %); and those of the control group ranged from 1.8 – 3.9 % (n = 105, mean 2.82 % & SD 0.43 %). The Hb A₂ level of the malaria patients was elevated statistically significantly than that found in 105 healthy controls (P < 0.05 at 95 % confidence limit).

Conclusion: From the study, we can conclude that one should remain conscious while interpreting laboratory tests in patients with malaria or patients from malaria endemic zone for β thalassemia trait which rely mainly on Hb A₂ level.

Keywords: Haemoglobin A₂, malaria, thalassemia, megaloblastic anaemia, High Performance Liquid Chromatography.

INTRODUCTION

As a result of the synthesis of different globin chains at different stages of life, there is a difference in the type of haemoglobin present in red cells between adult life and the fetal and neonatal periods. In adults, 96 - 98% of haemoglobin is haemoglobin A, which has two α chains and two β chains. A minor haemoglobin, haemoglobin A₂ (Hb A₂) has two α chains and two δ chains. A very minor haemoglobin in adults, but the major haemoglobin during fetal life and the early neonatal period, is haemoglobin F or fetal haemoglobin, which has two α chains and two γ chains. In the early embryo, haemoglobin is synthesized in the yolk sac and specific embryonic haemoglobins are produced - Gower 1 ($\zeta_2\epsilon_2$), Gower 2 ($\alpha_2\epsilon_2$) and Portland (or Portland 1, $\zeta_2\gamma_2$). Hb A₂ level is restricted to approximately 2.5% of total haemoglobin in healthy

adults. Hb A₂ and various other haemoglobins can be quantified with acceptable accuracy by High Performance Liquid Chromatography (HPLC), microcolumn chromatography, cellulose acetate electrophoresis followed by elution and spectrophotometry and capillary zone electrophoresis.¹

Since it was proved in 1957, Hb A₂ level elevation has frequently been used as a criterion for the diagnosis of β thalassemia trait in human population surveys. Some other factors are also considered to modify the Hb A₂ level temporarily including iron deficiency which decreases it, whereas it is increased in some cases of malaria & megaloblastic anaemia. Hb A₂ has also been found to be elevated in some cases of myelofibrosis, viral hepatitis, schistosomiasis and in recipients of fetal hematopoietic tissue.²⁻⁴

Many studies have been done to find the possible influence of malaria on the level of Hb A₂. These studies have produced divergent results. Since many surveys for the prevalence of β thalassemia have been made in areas with high prevalence of malaria such as sampling area of present study, it is important to find out whether malaria can modify Hb A₂ levels and, if so, to what extent. In present study, we have analysed Hb A₂ level of patient with malaria and thalassemia and then we have compared it to control group to conclude that whether Hb A₂ level was affected or not.

MATERIALS & METHODS

The present study was conducted at Sickle cell laboratory which is being operated under *Sickle Cell Anaemia Control Programme* run by Government of Gujarat in collaboration with non-government organizations. The present Sickle cell laboratory has been identified as tertiary center. In present study, data analysis has been done for samples of patients of tertiary hospital who were already diagnosed to have malaria by laboratory services of the same hospital. The control group & thalassemia group were selected from blood samples received for haematological investigations related to haemoglobinopathies. A confounding factor of megaloblastic anaemia was excluded by not including five samples with MCV > 100 fl. Five patients in malaria group who were already diagnosed to have thalassemia & haemoglobin E disorder were also excluded. Sample analysis has been done as per standard ethics & by maintaining confidentiality of test results.

Total 99 samples of malaria patients, 111 samples of β thalassemia trait patients & 105 samples of control group were tested. Samples were tested for complete blood count, DTT, blood group, haemoglobin electrophoresis at alkaline pH on cellulose acetate and HPLC on BIORAD VARIANT (Beta thalassemia short programme). The Hb A₂/F calibrator and two levels of controls (BIORAD) were analyzed at beginning of each run. Malaria parasites were looked for in the thick & thin films stained with Giemsa and the species of plasmodium were determined by experienced observers. Any doubtful smear for species identification was dealt with rapid malaria diagnosis kit.

The Null hypothesis was assumed that there was no difference in Hb A₂ level in malaria patients & control group and if any difference observed that was just because of different sample population. The p value of < 0.05 was used to consider difference as significant & to reject null hypothesis. The t test (two tailed distribution, two samples unequal variance) was used as hypothesis test with the help of Microsoft Excel 2010 software.

RESULTS

The Hb A₂ levels of the total 99 malaria patients were measured. Out of these, total ten patients (five

patients with MCV > 100 fl and five patients with haemoglobinopathies) were excluded from analysis. The Plasmodium species of 89 patients are shown in fig 1.

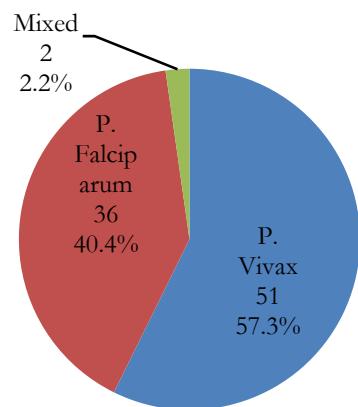


Fig: 1: Malaria Species (n=89)

Hb A₂ level in malaria group ranged from 1.9 – 4.0 % with a mean of 3.09 % & SD of 0.32 %; those of the β thalassemia trait group ranged from 4.0 – 7.3 % with a mean of 5.37 % & SD 0.74%; those of the control group ranged from 1.8 – 3.9 % with a mean of 2.82% & SD 0.43%. Hb A₂ level in malaria group was raised as compare to control group but not up to the extent seen in β thalassemia trait group.

The difference between the mean Hb A₂ levels of the 89 malaria patients and of the 105 controls was significant ($P < 0.05$, table 1). Of the 89 patients, 36 were infected with P. falciparum, 51 with P. vivax, and 2 with both the species. The levels of Hb A₂ classified according to the species of plasmodium also showed significant differences in comparison with those of control group (Table 1).

Tables 1: Level of Hb A₂ in malaria patients in comparison with that in healthy individuals

n	Hb A ₂		p value at 95% CI
	Mean	SD	
Control	105	2.82	0.43
Malaria	89	3.09	0.32
Malaria - P Falciparum	36	3.13	0.34
Malaria - P Vivax	51	3.07	0.32

The influence of anaemia on Hb A₂ level was also investigated in present study. The cut-off level considered for anaemia was 13 g/dl haemoglobin in males and 12 g /dl for females. In present study, 61 malaria patients were anaemic with mean Hb A₂ 3.08%, while 29 were not anaemic with mean Hb A₂ 3.10%. No significant difference was found between the Hb A₂ levels of the malaria patients with or without anaemia ($P = 0.84$). Furthermore, there was no significant correlation between the blood haemoglobin level and the Hb A₂ percentage. ($r = 0.05$).

DISCUSSION

Few studies have been done in past about influence of pathologic conditions besides thalassemia trait on Hb A₂ level. In present study, influence of malaria on Hb A₂ level has been investigated. Malaria has been selected as other pathology affecting Hb A₂ level as malaria is quite prevalent in this region & screening for thalassemia is being done using elevated level of Hb A₂ as a criterion. While conducting this study, certain samples from malaria patient who have already other pathology as confounding factors such as patients with macrocytic blood picture & already diagnosed cases of haemoglobinopathies have been eliminated.

The conclusions of various studies done in past for the influences of malaria on Hb A₂ level are shown in table 2. Out of these, four studies had concluded that malaria has significant effect on the level of Hb A₂ as compare to control group. One of these four studies, Wasi P et al found that malaria had negative impact on Hb A₂ & they concluded that Hb A₂ level was lower in patients with malaria as compare to healthy individuals.⁵ The rest of three studies conducted by Arends T et al, Lie InjoLuangEng et al & Isaacs A et al have demonstrated that Hb A₂ level were elevated in malaria patients as compare to control groups.⁶⁻⁸ The present study is also in accordance with these three studies. In present study we have analysed Hb A₂ level in 89 patients with malaria & compared it with Hb A₂ level in 105 healthy individuals & with thalassemia group of 111 patients. We concluded that the difference between the mean Hb A₂ levels of the 89 malaria patients and that of the 105 healthy individuals was significant ($P < 0.05$, table 1). There were no elevation in Hb A₂ level in malaria patients in study performed by Esan G J F et al, Wilcox M C et al & Van Ros et al.^{3,9,10}

Iron deficiency anaemia & thalassemia are two very important conditions which affect Hb A₂ level. The blood picture in both of these conditions is that of microcytic anaemia. The iron deficiency anaemia can be

differentiated from thalassemia with the help of mentzer index. It is calculated from the results of a complete blood count. If the quotient of the mean corpuscular volume divided by the red blood cell count is less than 13, thalassemia is more likely. If the result is greater than 13, then iron-deficiency anaemia is more likely.¹¹ Mentzer index was applied to eliminate possible inclusion of patients with β thalassemia traits as well as at the same time to include patients with iron deficiency anaemia in control as well as the study group with malaria.

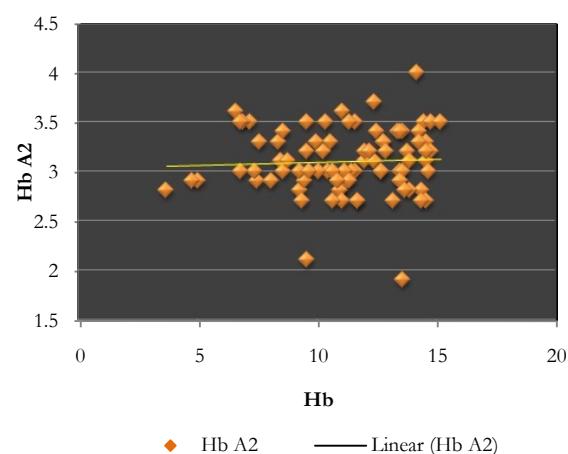


Fig 2: Correlation between Hemoglobin level and Hb A₂

The effect of anaemia on Hb A₂ level has been also investigated in patient with malaria in present study. No significant difference was found between the Hb A₂ levels of the malaria patients with or without anaemia ($P = 0.84$). Furthermore, there was no significant correlation between the blood haemoglobin level and the Hb A₂ percentage ($r = 0.05$, figure 2). These both finding of present studies are consistent with that of study done by Van Ros et al.³

Table 2: Various studies for Hb A₂ level in malaria patients

	Sample size	Mean (%)	SD (%)	Range	Hb A ₂ comparison with control	p value
Arends T ⁶	8	3.41		3.0-4.0	increased	< 0.001
Lie-InjoLuangEng et al ⁷	42	3.32	0.69	1.9-5.1	increased	< 0.02
Isaacs A et al ⁸	19	4.11	0.31	1.6-7.3	increased	< 0.05
Esan G J F et al ⁹	81	2.39	0.49	1.50-3.56	ND*	> 0.20
Wilcox M C et al ¹⁰	169				ND	> 0.30
Wasi P et al ⁵	61	2.43	0.37	1.63-3.17	decreased	< 0.005
Van Ros et al ³	94	2.76	0.51	1.9-4.6	ND	> 0.40
Present Study	89	3.09	0.32	1.9-4.0	increased	< 0.05

*No difference

No significant difference in Hb A₂ levels due to different species of plasmodium (P vivax and P falciparum, $p = 0.4$ at 95% CI) was found. As we have good proportion of both species in present study, we can conclude that species variations in plasmodium is not confounding factor for affecting Hb A₂ level.

CONCLUSION

As mentioned earlier, there are different methods available for quantitative estimation of Hb A₂ level.¹ Out of all these, HPLC is one of the best methods for quantification. One must take into consideration method used for quantification also

before concluding that whether Hb A₂ level is elevated in malaria patients or not. From present study we can conclude that malaria may have influence on Hb A₂ level & patients with malaria may have transient elevated level of Hb A₂. The reason behind divergent result of present study from some previous studies may be because of difference in study designs, quantification methods of Hb A₂& sampling population. We can conclude that one should remain conscious while interpreting laboratory tests in patients with malaria or patients from malaria endemic zone for β thalassemia trait which rely mainly on Hb A₂level.

The present study has some limitations like molecular analysis was not done for all patients with elevated Hb A₂ level to rule out β thalassemia trait and follow up studyof malaria group for repeat Hb A₂ level was not done. The authors of present study plan to design a study with inclusion of molecular analysis in future for more accurate analysis.

Conflicting Interest: Non declared

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