

Relative Protection from Ischaemic Heart Disease in Beta-Thalassaemia Carriers

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ABSTRACT

Objective: To compare the frequency of beta thalassaemia trait in individuals with Ischaemic Heart Disease (IHD) and a control population without IHD.

Study Design: Case control study.

Place and Duration of Study: Department of Haematology, Armed Forces Institute of Pathology (AFIP), Rawalpindi, from September 2007 to May 2009.

Methodology: Using non-probability consecutive sampling, a total of 544 subjects were selected, including 272 IHD patients and an equal number of age and gender matched normal controls. The subjects were tested for the presence of β -thalassaemia trait by performing their blood counts, haemoglobin electrophoresis and Haemoglobin A₂ (HbA₂) estimation. Proportions were compared using chi-square test. Odds ratio was also calculated.

Results: The frequency of β -thalassaemia trait was determined in IHD patients and was compared to the frequency in normal Pakistani population. Six out of the 272 control subjects (2.2%) had β -thalassaemia trait and one of the control subject had Haemoglobin D trait. In contrast, none of the 272 IHD patients had β -thalassaemia trait. The calculated odds ratio was less than 1, which shows a significant negative association of β -thalassaemia trait with IHD. The difference in the frequency of β -thalassaemia trait in the two groups was statistically significant ($p=0.033$).

Conclusion: The results suggest that β -thalassaemia carriers have some protection against IHD, though it is not an absolute cardio protection due to the role of other risk factors in IHD. This beneficial information may be communicated to the concerned individuals in their counselling sessions and as part of general awareness on thalassaemia.

Key words: β -Thalassaemia trait. Ischaemic heart disease (IHD). Counselling. Cardio protection.

INTRODUCTION

Beta thalassaemia is one of the most common genetic haemoglobin disorders of the world, with a high prevalence in the Mediterranean region, Middle East, Indian subcontinent and South East Asia.¹ In Pakistan, it is the most common inherited disorder with a carrier rate of 5%, whereas the incidence of β -thalassaemia major is 1.2/1000 births.² The most appropriate approach towards this disorder is its prevention. The preventive aspect comprises of three important pillars, which include screening for thalassaemia carriers, prenatal diagnosis and genetic counselling. Genetic counselling, if done in an appropriate manner can contribute immensely towards prevention of this disorder. Critical issues like marriage options, importance of consanguinity, screening of spouse and family, prenatal diagnosis and termination of pregnancy, have to be explained and discussed in detail. These facts make the counselling of thalassaemia carriers a challenging task, yet sharing of some positive beneficial

information in these sessions can be helpful in confronting these counselling issues. One such fact is the epidemiologically confirmed protection against falciparum malaria in the thalassaemia carriers,³ the exact nature of this protection is not known, but various mechanisms like increased phagocytosis of parasitized red cells,⁴ have been postulated.

Similarly it has been reported that heterozygous β -thalassaemia confers some protection against ischaemic heart disease (IHD). IHD is a condition with high prevalence,^{5,6} morbidity and mortality. The cardio protective effect may be due to lowering of low density lipoprotein cholesterol (LDL) levels in β -thalassaemia carriers.⁷ This results from increased uptake of LDL cholesterol by the bone marrow,⁸ due to the lifelong increased cholesterol requirements to maintain increased erythroid proliferation occurring in thalassaemia carriers.⁹ In addition, reduced blood viscosity due to slight anemia and sustained life long microcytosis in thalassaemia carriers also contributes towards the said cardio protection.¹⁰

If this hypothesis is correct, then the frequency of β -thalassaemia trait should be low in IHD patients as compared to the non IHD population. Therefore, this study was conducted to determine the frequency of β -thalassaemia trait in IHD patients and compared it with that of the normal controls. The results of this study

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would be helpful in validating or refuting the cardio protection seen in β -thalassaemia carriers. The negative association, if established could prove to be a beneficial tool in the counselling of β -thalassaemia carriers, while curbing their negative feelings at the same time.¹¹

METHODOLOGY

This case control study was conducted at the Department of Haematology, Armed Forces Institute of Pathology, Rawalpindi. The study was conducted, from September 2007 to May 2009. The study included a total of 544 subjects selected by non probability consecutive sampling, including 272 IHD patients diagnosed at Armed Forces Institute of Cardiology (AFIC), Rawalpindi. An equal number of gender and age matched normal individuals visiting AFIP were also studied as normal controls.

After patient reassurance and consent, 5 ml of venous blood was drawn from antecubital vein by aseptic technique. Ethylenediamine tetra-acetic acid (EDTA) was used as an anticoagulant; at concentration of 1.5 ± 0.5 mg/ml. Samples collected at AFIC were also transported to AFIP for required laboratory investigations.

Blood counts were performed on Sysmex KX-21 automated haematology analyzer. All individuals with Mean corpuscular volume (MCV) ≤ 75 fl and/or Mean corpuscular haemoglobin (MCH) ≤ 25 pg were investigated further by haemoglobin electrophoresis and haemoglobin A₂ estimation.

Hemolysate was prepared by mixing saline washed red cells with distill water and carbon tetrachloride, followed by centrifugation at 3000 rpm for 15 minutes. Haemoglobin electrophoresis of the supernatant lysate was performed on cellulose acetate membrane soaked in Tris EDTA borate buffer having pH 8.9.¹² Normal and positive controls were also applied on the same electrophoresis strip. The cellulose acetate strip was then stained with 0.2% ponceau S stain for 15 minutes. Excess stain was cleared in acetic acid. Visual assessment of the bands of Haemoglobin A₂ of the patients was done, by comparing with normal and positive controls. Haemoglobin A₂ estimation was done at the end by elution of the haemoglobin bands and measurement of the absorbance by spectrophotometer at wavelength of 416 μ m.

Haemoglobin A₂ levels were calculated by using the following formula¹²:

$$\% \text{HbA}_2 = \frac{\text{Absorbance of haemoglobin A}_2 \times 100}{\text{Absorbance of haemoglobin A}_2 + (\text{absorbance of haemoglobin A} \times 4)}$$

All individuals with $\text{HbA}_2 \geq 3.5\%$ were diagnosed as β -thalassaemia trait.¹³ It is worth noting that a few factors can affect HbA_2 levels, as it has been reported

that HbA_2 may be increased in patients having HIV infection,¹⁴ and rarely even normal individuals may have raised HbA_2 levels.¹⁵ Odds ratio was calculated to compare the frequency of β -thalassaemia trait in the two groups, chi-square test was applied.

RESULTS

Out of the 272 IHD patients, 221 were males (81.2%) and 51 were females (18.8%), with a male to female ratio of 4.3:1. In the control group the number of males and females were also kept same for gender matching. Median age in the IHD patients was 63 years, whereas in the control population it was 61.5 years. The mean haemoglobin in the IHD patients was 12.5 g/dl (± 2.37), and in the control group it was 12.7 g/dl (± 2.02). The mean MCV in the IHD patients was 93 fl (± 8.16), and in the control population it was 84 fl (± 8.17). The mean MCH in the IHD group was 28.5 pg (± 2.80) and in the controls it was 27.15 pg (± 3.4).

In the control group, 28 subjects (10.3%, 95% Confidence Interval [CI] = 6.6-14.1%) had a low MCV/ MCH, and 6 individuals (2.2%, 95% CI= 0.5 – 3.96%) had HbA_2 level of $> 3.5\%$, and were diagnosed as β -thalassaemia carriers. All the carriers in the control group were males, probably an incidental finding due to the male predominance in the group. One of the female in the control group (0.4%) was haemoglobin D trait, a clinically asymptomatic condition. In the IHD group, 14 patients (5.14%, 95% CI = 2.8 – 8%) had a low MCV/ MCH, but none of the patients had β -thalassaemia trait. The results in the IHD patients could be affected by the fact that majority (81%) of the patients were males, as this fact is important because it is being debated that whether the cardio protection seen in thalassaemia carriers is only restricted to males.¹⁶

The calculated odds ratio was less than 1, which shows a significant negative association between β -thalassaemia trait and IHD. The difference in the frequency of β -thalassaemia trait in the two groups was found to be statistically significant, as the p-value was less than 0.05 (p=0.033). Results of the comparison in the frequency of β -thalassaemia trait in the two groups are shown in Table I.

Table I: Comparison in the frequency of β -thalassaemia trait in IHD and non IHD groups.

Group	β -Thalassaemia trait		Total
	Yes	No	
A	6 (2.2%)	266 (97.8%)	272
B	0 (0%)	272 (100%)	272
Total	6 (1.1%)	538 (98.9%)	544

Group A: Normal control population; Group B: IHD patients; P-value = 0.033 (The observed difference is statistically significant).

DISCUSSION

β -thalassaemia carriers though clinically asymptomatic, still face stigmatization and social problems due to misconceptions and lack of knowledge and awareness

about the condition. Thalassaemia carriers can face problems at the time of their marriage and the carrier status can even become a hurdle in adopting certain professions with stringent health criteria. The possibility of birth of a thalassaemia major child is also a matter of concern for them. Fortunately the prevention programmes against thalassaemia based on prenatal diagnosis and screening to identify carriers can solve most of the problems. Even then the above mentioned issues can be a source of mental tension in the thalassaemia carriers. Such tensions may be alleviated by counselling and increasing the awareness about the condition. Counselling can be made more effective and productive by communication of the beneficial information linked to the condition, like the well established fact about the protection against malaria in thalassaemia carriers.³

Another beneficial information includes protection against IHD. If this is correct then the frequency of β -thalassaemia trait should be lower in IHD patients as compared to the non IHD population. Therefore, this study was aimed to test the hypothesis that "frequency of β -thalassaemia is low in patients with IHD than in individuals without IHD". The hypothesis was supported by the results of this study. The frequency of β -thalassaemia trait in the control group was well above the frequency found in IHD patients. This difference was statistically significant and the odds ratio of < 1 established a significant negative association between thalassaemia trait and IHD. The male predominance in the study population could be incidental, but a contributing factor could be the female under-representation in seeking medical attention in our subcontinent.

Only few studies have been done internationally on this subject. This study is the first of its kind in Pakistan, as no local data was available for comparison of results. The results are almost similar to those reported by other international studies by Crowley *et al.*¹⁶ Gallerani *et al.*¹⁷ Tassiopoulos *et al.*¹⁰ and Wang *et al.*¹⁸ All these international studies have validated the cardio protection in β -thalassaemia carriers. Only one international study by Hashemi *et al.* found no statistically significant difference in the frequency of thalassaemia trait in the two groups.¹⁹

The results obtained in the IHD group did not allow to verify the facts regarding the cardio protection being restricted to men, and a higher mean age of IHD presentation in carrier males, as highlighted by Gallerani.¹⁷

Other postulated protective effects of heterozygous β -thalassaemia against cerebrovascular accidents (CVA)²⁰ and hypertension,²¹ and its association with low cholesterol levels, should also be studied and validated. All these facts, if established, would be greatly helpful in

enhancing the effectiveness of genetic counselling of thalassaemia carriers, making them more receptive towards other preventive modalities like carrier screening and prenatal diagnosis.

CONCLUSION

The results of the study suggest that β -thalassaemia carriers are protected against ischemic heart disease, though it may be termed a relative protection, due to the role of other risk factors associated with IHD. This is very useful information and it may be communicated to β -thalassaemia carriers in their counselling sessions and at the same time the information may be propagated as part of the general awareness on thalassaemia.

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