The genetic basis of the clinical diversity of the Trans-Resveratrol therapy in beta-thalassemic and sickle cell anaemia patients.

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Abstract Trans-Resveratrol responses were influenced by Several genetic, non-genetic and pharmacological factors in different early studies. The response to Trans-Resveratrol is significantly different among good, moderate and non-responders irrespective of the IVS I-5 (G→C), the common beta mutation here and even among other β^0 or β^+ thalassaemia mutations. It has been shown that even among good responders in some cases (8.39 %) patients are not showing high HbF values. (< 20% HbF values are taken).

We studied among 220 patients that Trans-Resveratrol therapy completely replace blood transfusion in Eastern part of India. The predominant β-thalassemia defect is the IVSI-5(G→C), the most frequent β0-thal mutation in the area. This mutation is found in 87.66% of the CR patients and in 86.66% of the PR / NR patients. The next represented defects are the β+-thalassemia mutations like Cod 8/9, Fr. 41/42, Cod 15, Cod 30 etc. The distribution of the IVSI-5 (G→C) genotype among the CR, PR / NR. The common IVSI-5 (G→C) mutation in beta thalassemia patients (either in homozygous or heterozygous form) is present in 31.5% among the good responders (CR) while it is present in 66.66% among PR / NR (moderate responders / non-responders) and in HbE-beta patients present in 73.28% among good responders (CR) while in 33.33% among moderate / non responders (PR / NR).

Key words: Resveratrol, β-thalassemia, HbF , Blood transfusion, Good responder, Moderate responder, Non responder.

Introduction

The β-thalassemias are characterized by a very heterogeneous group of inherited mutations causing abnormal expression of globin genes, leading to total absence or quantitative reduction of synthesis of β-globin chains (1–3). This disease is frequent in the Mediterranean area, Middle East, Africa and Asia. More than 200 different mutations have been identified in β-thalassemia patients, including deletions of the β-gene region, stop codons leading to premature termination of a non-functional β-globin chain, mutations suppressing correct maturation of the β-globin RNA precursor, most of all need regular blood transfusions. (1–3,4,5).

Resveratrol is a ribo-nucleotide reductase inhibitor, much more effective than hydroxyurea (HU), a molecule that has been used clinically as therapeutic agent for the treatment of Sickle cell anaemia, a haemoglobinopathy characterized by the polymerization of a mutated form of adult haemoglobin (HbS) leading to erythrocyte sickling which plays an important part in the disease morbidity. Hydroxyurea has proven its clinical benefit as it can increase fetal haemoglobin (HbF) levels in the red cells of sickle cell patients inhibiting HbS polymer formation.

Hence, we study that the variations of nucleotide in the β-globin gene cluster and their association with the Trans-Resveratrol response so that we could explore the genetic basis of the clinical diversity of the Trans-Resveratrol therapy in beta-thalassemic and sickle cell anaemia patients.

Again, we may assume that a large number of the thalassaemic patients in Eastern India have a **molecular background favorable to Trans-Resveratrol response**, because, during the one year of treatment (followed by **Thalassaemia Foundation**) we did not observe any significant problems regarding drug compliance and no myelogenic or clinically adverse events occurred. This in agreement with other long-term clinical trials which reported no significant increases in secondary malignancy following Trans-Resveratrol therapy.

Materials & Methods

Study groups:

Patients with HPLC-screened documented Sickle cell anaemia, S-beta thalassemia, beta thalassaemia, HbE thalassaemia, HbE-beta thalassaemia, HPFH genotypes have been considered in this primary analysis.

Collection of Sample: Sample was collected from OPD of Thalassaemia Foundation, Kolkata. Total 220 patients were evaluated. Among which 140 patients with Hb-E-beta and 69 patients with Beta and HPFH and 11 patients with other hemoglobinopathies were observed.

Molecular Analysis:

Samples for DNA isolation have been collected. DNA has been extracted and genotyping has been done by ARMS-PCR for the common beta mutations of this region, like IVSI-5(G→C), Cod 8/9, Fr.41/42, Cod15, Cod26 and also for Cod6 (Sickle cell anaemia).

Fetal hemoglobin studies

Hb variants' (HbA / HbA2 / HbF & others) levels was estimated by HPLC (High Performance Liquid Chromatography) (Bio-Rad, USA). Estimation of HbF was also done by using HPLC method.

Result

Clinical profile

We were able to classify three categories of response: a Complete Response (52.2%) in patients who can able to maintain at an average Hb level of 6-9 gm/dL without blood transfusion, in this group 12.3% patients are without any previous H/O blood transfusion, others shifted from monthly blood transfusion dependency to a stable transfusion-free condition; a Partial Response (18.2%) in patients who remained transfusion dependent but at longer intervals (2-3 months or more), and Non response (15.9%) in patients who, after more than one year of treatment, remained at the same level of transfusion dependency.

Molecular analysis

Samples for DNA isolation have been collected. DNA has been separated for mutation analysis by ARMS-PCR for common beta thal mutations of India. Patients were studied at the molecular level for their β-globin gene mutations.

As expected in this Eastern part of India, the predominant β-thalassemia defect is the IVSI-5(G-C), the most frequent β0-thal mutation in the area. This mutation is found in 87.66% of the CR patients and in and 86.66% of the PR / NR patients. The next represented defects are the β0-thalassemia mutations like Cod 8/9, Fr. 41/42, Cod 15, Cod 30 etc. The distribution of the IVSI-5 (G→C) genotype among the CR, PR / NR categories is summarized in **Table 1**.

Table 1 : Categories of β-thalassaemia genotypes responding with Trans – Resveratrol therapy

Genotype β/β	*CR (%)*	*PR / NR(%)*
IVSI-5 (G→C) / IVSI-5 (G→C)	**14.38**	**53.33**
IVSI-5 (G→C) / cd 26	**73.28**	**33.33**
IVSI-5 (G→C) / cd 6	**----**	**----**

The common mutation in beta thalassaemic patients (either in homozygous or heterozygous form) i.e IVSI-5 (G→C) is present in 31.5% among the good responders (CR) while it is present in 66.66% among PR / NR (the moderate responders / non-responders) and in HbE-beta patients present in 73.28% among good responders (CR) while in 33.33% among moderate / non responders (PR / NR). (Table 2).

Table 2: β-thalassaemia genotypes *(β/β)* responding with Trans – Resveratrol therapy

Beta Mutations	Good Responder(%)	Non Responder(%)
Beta Homozygous		
IVSI-5 (G→C) / IVSI-5 (G→C)	**14.38**	**53.33**
Beta Heterozygous		
IVSI(G→C) / cd8/9	**-----**	**------**
IVSI-5 (G→C) / Fr.41/42	**17.12**	**13.33**
IVSI-5 (G→C) / cd15	**-----**	**------**
HbE-beta		
IVSI-5 (G→C) / cd 26	**73.28**	**33.33**

The response to Trans-Resveratrol is equal in males and females and the age distribution is not significantly different in the three response categories. Similarly, no significant difference in response was found between splenectomized and non-splenectomized patients.

Discussion

Since Resveratrol has been demonstrated to inhibit ribonucleotide reductase with an efficiency higher than HU (6)**.** Rodrigue et al. (7) found that resveratrol possesses similar properties to HU toward erythroid differentiation. They firmly demonstrated that resveratrol induces differentiation of K562 cells and augmentation of HbF in erythroid precursor cells. Comparative analyses demonstrated that resveratrol, as HU, inhibits intracellular adhesion molecule-1 (ICAM-1) and VCAM-1 expression by endothelial cells. In addition, resveratrol possesses other properties similar to HU, including induction of nitric oxide synthase in cultured pulmonary endothelial cells and inhibition of human platelet aggregation in vitro. Interestingly, resveratrol exhibited minimal toxicity on normal hematopoietic cells, as suggested by Cle´ment et al. (8)**.**

These data strongly indicate that resveratrol is a strong inducer of HbF and a selective stimulator of the expression in β-globin genes. Again, since the effect of Resveratrol on thalassaemics is not completely understood by large number of applications and we have a very good number of patients who have not responded on HU therapy though patients having all the positive factors in their genetic background (one of which is already possessing high HbF conc. in their RBCs) known yet today, hence we want to study the genotypes of patients who responded on resveratrol but previously not on HU. So the hypothesis is that to find the extra/different molecular background for which the patients can get a benefit may be in their increase of Mean Cell Haemoglobin (MCH) or in having betterment in the RBC morphology.

In our present study we observed that whether HbF level has any relation to beta variants responding to Trans-Resveratrol therapy, it has been shown that even among good responders in some cases (8.39 %) patients are not showing high HbF values (< 20% HbF values are taken).

We observed that Trans-Resveratrol therapy completely replace blood transfusion in 88% cases (79.5% HbE-beta intermedia, 13.6% in beta thalassaemia major and 6.8% in other haemoglobinopathies like HbE disease & Sickle Cell anaemia).

According to Bianchi et al, when erythroid precursor cells from normal subjects were treated with increasing concentrations of resveratrol and analysis of accumulation of globin mRNA sequences was performed by quantitative RT-PCR, a clear increase in accumulation of γ-globin mRNA content was found. Increase in accumulation of α-globin and β-globin mRNA was much lower. Taken together these data strongly indicate resveratrol as a strong inducer of HbF and a selective stimulator of the expression in γ-globin genes.(9)

Conclusion:

I. Trans-Resveratrol responses were influenced by Several genetic, non-genetic and pharmacological factors in different early studies. The response to Trans-Resveratrol is significantly different among good, moderate and non-responders irrespective of the IVS I-5 (G→C), the common beta mutation here and even among other β^0 or β^+ thalassaemia mutations.

II. We studied that any relation has present between HbF level and beta thalasaemic variants responding to Trans-Resveratrol therapy, it has been shown that even among good responders in some cases (8.39 %) patients are not showing high HbF values (< 20% HbF values are taken).

III. Among 220 patients we studied that 88% cases (79.5% HbE-beta intermedia, 13.6% in beta thalassaemia major and 6.8% in other haemoglobinopathies like HbE disease & Sickle Cell anaemia). Trans-Resveratrol therapy completely replace the blood transfusion.

Funding statement: This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors. All the research work done by the affiliated institution funding.

Competing Interests Statement: The authors declare that they have no competing interests.

Data Sharing Statement: We cannot share any unpublished data with other laboratory or person.

Patients Consent Statement: The signed consent from all the patients were taken before test was performed and kept them as official documents. In case of any unusual condition it will be presented in front of the concerned person.

Acknowledgement

Author acknowledges Institute of Genetic Medicine and Genomic Sciences for funding and affiliation. We are also thankful to other laboratory members and other associated persons of IGE and IGMGS for their enthusiastic participation.

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