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RESEARCH ARTICLE

RESVERATROL AS AN ALTERNATIVE TO REGULAR BLOOD TRANSFUSION- A NEW FETAL HAEMOGLOBIN INDUCER FROM NATURAL WORLD

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Abstract

Recent molecular studies of fetal hemoglobin (HbF) regulation have shown promise for the development of clinical HbF inducers to be used in patients with β-thalassemia and sickle cell disease. However, while numerous promising inducers of HbF, have been studied at past in β-thalassemia patient with limited success resulted no universally effective agents. Increased production of fetal hemoglobin (HbF) can ameliorate the severity of both β-thalassemia and sickle cell disease (SCD), the major disorders of β-hemoglobin. The defective production of the β-globin molecule in patients with β-thalassemia can be compensated for by an increase in the production of the β-like globin molecule, γ-globin, which pairs together with α-globin chains to form HbF. Here we examined the clinical studies of one HbF inducer Trans-Resveratrol and found Complete Response (52.2%), Partial Response (18.2%) and Non response (15.9%) in patients who, after more than one year of treatment, remained at the different level of transfusion dependency with extended transfusion intervals. The present study is to provide a resource that will be valuable for the design of future studies of HbF inducers in β-thalassemia. According to our knowledge and literature review, probably this could be the first report for resveratrol clinical trial in eastern Indian population.

Introduction:-

Resveratrol (3,4',5-trihydroxystilbene) belongs to a class of poly-phenolic compounds called stilbenes **(**1**)** which is effective in response to stress, injury, fungal infection, or ultraviolet (UV) radiation **(**2**).** Resveratrol is a fat-soluble compound that occurs in a *trans* and a *cis* configuration in combination to glucose forming glucosides. Resveratrol-3-*O*-beta-glucoside is called piceid **(**3**).** Literature study revealed that, Scientists became interested in exploring potential health benefits of resveratrol in 1992 when its presence was first reported in red wine **(**4**),** and more recently, reports on the potentiality of resveratrol to inhibit the development of cancer **(**5**)** and extend lifespan **(**6**)** in cell culture and animal models have continued to generate scientific interest .From 2005 until the middle of 2010, there have been more than thousands new studies on cells, animals, and humans. Not a single commercially available drug was known to medical science which had the wide range of potential preventative, therapeutic, and quality of life enhancement properties as like as resveratrol. It has been shown to inhibit cancer, kill bacteria, viruses

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and fungal infections, extend life span in animals, improve energy production in cells, quench free radicals, increase glucose tolerance in diabetics, improve cardiac function, enhance physical and mental fitness and concentration, repair damage of DNA, prevent cell damage from nuclear radiation, and much more.

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 **(**7-9**).** 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-globin 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. **(**7-11**).** In addition to 'direct-costs', blood transfusions require accurate monitoring of the safety of the product which involves expensive technologies. As far as alternative therapeutic approaches are concerned, gene therapy and bone marrow transplantations are very promising strategies but they are expected to be useful for only a minority of patients, selected on the basis of biological/genetic parameters and the economic possibility of affording these therapies. Pharmacological therapy including possible exploitation of HbF inducers, is expected to be crucial. Induction of HbF in patients affected by β-thalassemia and sickle cell anemia (SCA) has been suggested as a very promising approach for the conversion of those patients to an independency from blood transfusion **(**12-19**).** On this context, the present study was conducted to detect the efficacy of resveratrol as a potent HbF inducing agent.

Materials and 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. Age distribution of Patients, Types of thalassaemia, duration of blood transfusion, duration of treatment of resveratrol was clearly depicted in Table-1, 2, 3 and 4 respectively.

Table 1:- Age distribution of patients.

Age group	Percent(%)
1-25 years	94.5
25-50 years	5.5

Table 2:- Types of Thalassaemia

Types of Thalassaemia	Percent(%)
HbE-Beta (Intermedia)	71.42
HbE	0.7
Beta	26.19
HbSS	0.7
HbD-S	0.7

Table 3. Duration of Blood Transfusion.

Duration	Percent (%)
monthly	17.5
< 1month	5.6
2 months	5.6
> 2 y – 5 y	14.2
10 y	0.8
No Blood Transfusion	56.3

Table 4:- Duration of Treatment on Resveratrol.

Trans-Resveratrol therapy continued	Percent(%)
More than 1 year	35.19
More than 6 months	13.89

More than 3 months	24.07
Less than 3 months	6.48
No treatment	20.37

Collection of Sample:-
Sample was collected from OPD of Thalassaemia Foundation, Kolkata. Total 220 patients were evaluated. Among which 142 patients with Hb-E and 69 patients with Beta and HPFH and 11 patients with other hemoglobinopathies were observed.

Hematological Analysis:-
Analysis (Hb level / Total WBC / Mean Cell Volume / Mean Cell Hemoglobin / Mean Cell Hemoglobin Concentration / Red Cell Distribution Width / Hematocrit) was done by Automated analysis (Cell Counter : Medonic 530, EMerck).

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.

Biochemical Analysis:-
Liver Function test (Serum Alanine aminotransferase concentration / Serum Aspartate aminotranseferase Concentration / Total protein levels) & Renal Function test (Serum Creatinine concentration) was performed by Biochemical Analyser [Microlab 300, EMerck].

Result:-

The pre-treatment and post-treatment hematological analysis and fetal hemoglobin analysis about 87 patients with resveratrol therapy revealed that there was significant response in hematological parameters for the increase of transfusion time against control. Different blood parameters and their pre-treatment and post-treatment values in beta and E-beta thalassaemia patients were clearly depicted in **Table-5** and **Table-6.** The toxicity and side effect of resveratrol was evaluated by liver function test (LFT) and the bilirubin, SGOT and SGPT value against control were clearly depicted in **Table -7**. Baseline evaluation (clinical and biochemical) of all the patients result shows that, there was ***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; ***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. **[Table 8]**

Table 5:- Different blood parameters and their pre-treatment and post-treatment values in Beta thalassaemia patients

Blood Parameters	Control Initial	Control Final	Pre Therapy	Post Therapy
HbF	82.5±1.36	83.6±2.30	25.36±2.36	55.69±2.36*
HbA2	4.8±1.20	4.2±1.30	9.4±2.60	6.34± 1.25 *
HbA	60.23±2.36	62.35±1.20	6.36±3.60	10.32± 2.36 *
Hb	7.8±2.30	8.5±0.25	5.26±1.77	8.82 ±2.31 *
MCV	80.2±2.36	83.4±1.36	71.68±2.39	79.44 ± 1.07 *
MCH	31.0±1.23	32.0±0.69	24.48± 2.30	27.84±1.08 *
MCHC	38.7±1.36	38.4±2.36	32.66 ± 2.49	37.06 ± 2.07*
Rdw	16.6±1.25	16.36±1.20	42.76±1.20	38.76±2.57 *
Hct	20.1±0.56	22.2±1.30	19.64 ±2.86	24.24±1.55 *

Standard deviation was done in all the result, *Significant at P<0.05 against Control Final.

Table 6:- Different blood parameters and their pre-treatment and post-treatment values in E-beta thalassaemia patients

Blood Parameters	Control Initial	Control Final	Pre Therapy	Post Therapy
HbF	38.94±1.77	52.2±1.50	30.6± 01.36	39.18±5.01*
HbA2	53.44±1.48	43.4±1.25	44.76±5.83	55.34±4.59 *
HbA	4.74±3.32	4.4±1.30	3.72±1.47	7.57±2.70 *
Hb	8.61±0.93	8.31±1.03	5.87±1.17	7.17±0.89 *
MCV	63.31±1.60	63.25±1.78	62.83±2.75	65.28±1.25 *
MCH	22.86±2.59	23.5±2.92	20.72± 2.64	25.87±1.72 *
MCHC	36.1±0.77	35.91±0.46	32.78±0.58	35.56±1.98 *
Rdw	30.03±1.68	30.9±1.57	34.10±2.61	29.40±2.26*
Hct	23.91±2.69	22.96±3.07	16.87±0.72	20.26±1.53*

Standard deviation was done in all the result, *Significant at P<0.05 against Control Final.

Table 7: Toxicity/Side effect evaluation of Beta & HbE/Beta thalassaemia patients on Trans-Resveratrol by Liver Function Test

Type of Thalassaemia	Bilirubin (mg/dl)	SGOT (U/l)	SGPT (U/l)
Beta	2.07±2.5	20.0±3.6	30.5±3.5
E/Beta	2.25±2.3	22.6±2.3	28+-6.3
Control	1.50±0.5	<30	<40

**** Standard deviation was done in all the result**

Table 8:- Distribution of patients in different categories of response

Groups of different categories	n (%)	HbE-beta (n=142)	Beta/HPFH (n=69)	Haemoglobino-pathies (HbE, Sickle etc) (n=11)
COMPLETE RESPONSE				
GROUP-I	**88 (%)**	Female=24 (%)	Female = 5 (%)	Female = 5 (%)
(withdrawal of BT)		Male = 46 (%)	Male = 7 (%)	Male = 1 (%)
GROUP-II	**27 (%)**	Female = 9 (%)	Female = 2 (%)	Female = 0 (%)
(No H/O BT)		Male = 12 (%)	Male = 4 (%)	Male = 0 (%)
NON RESPONSE	**35 (%)**	Female = 2 (%)	Female = 5 (%)	Female = 0 (%)
GROUP-III		Male = 6 (%)	Male = 22 (%)	Male = 0 (%)
PARTIAL RESPONSE	**40 (%)**	Female = 9 (%)	Female = 9 (%)	Female = 0 (%)
GROUP-IV		Male = 11 (%)	Male = 10 (%)	Male = 1 (%)
CONTROL GROUP	**32 (%)**	Female = 9 (%)	Female = 2 (%)	Female = 2 (%)
(without HU)		Male = 14 (%)	Male = 3 (%)	Male = 2 (%)

Discussion:-

Over the last few years a substantial number of medical schools and research institutions have undertaken studies of resveratrol's ability to prevent or treat disease in humans. The number of such clinical trials is increasing day by day and mostly done on diseases like diabetes, heart disease and thalassaemia. In Thalassemia, either very few or no red blood cells being produced by the bone marrow after infancy. The treatment is monthly whole blood transfusions and the use of a drug which is extremely toxic and cannot be used safely with children. The disease dramatically impacts the sufferers' quality of life and often results in death around the age of puberty as it is more common in less developed countries where it is virtually impossible for anyone other than the very wealthy to obtain regular supplies of clean whole blood for the required transfusions. So, the fatality rate is high. Even if the patient is able to obtain monthly transfusions and is able to afford the drugs to treat the disease, he or she is constantly anemic and lacking of energy. After the discovery of Transmax resveratrol, the concentrated pure resveratrol supplement used by researchers in most clinical trials, was able to stimulate the production of embryonic red blood cells, the type that are produced when a baby is still in the mother's womb.

Resveratrol has been efficiently inhibiting ribonucleotide reductase as it possesses similar properties to HU toward erythroid differentiation. Rresveratrol 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. **(**20**).**

In this present study, pre-treatment and post-treatment of resveratrol and evaluation of blood CBC parameters in patients with beta and E-beta thalassaemia shows three categories of response: 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); 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), we observed increase of most of the CBC parameters which strongly indicate that resveratrol is a strong inducer of HbF and a selective stimulator of the expression in β-globin genes.

According to Bianchi et al, when erythroid precursor cells from normal subjects were treated with increasing concentrations of resveratrol, 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. **(**21**)**

Conclusion:-

For HbF induction therapy to become part of the standard management for patients with β-thalassemia, there needs to be a great deal of work from both basic scientists and clinical researchers. Though several genetic, non-genetic and pharmacological factors reported to influence the Trans-Resveratrol response in different early studies, the response to Trans-Resveratrol is significantly different among good, moderate and non-responders among β^0 or β^+ thalassaemia mutations. To study 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). In our study, Trans-Resveratrol therapy completely replaces 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). Hence it is evident that some other element associated with beta globin gene framework which could related to good response to Trans-Resveratrol, for which further studies required.

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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.

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