B-8 Vaidhya
Anushakti Nagar
Bombay-400085
India

9th Feb. 1991

Dear Dr. Garfield,

It may seem strange that after 3 years of silence, I am again approaching you with the same request and with added fervour.

If you recall, the reason for my letter to you was my uncle Mr. P.K. Subramaniam. Today my uncle is no more. He passed away on 10th Dec. 1990. I thought this life story may be an inspiration to young scientists all over the world. Way back in 1987 I wrote to you, explaining the possibilities of bringing out an editorial on him. You replied favourably, and wanted some typed copies of his publication and other relevant details. Since I myself was passing through a difficult phase then due to an accident, I couldn't comply with your request in the format you wanted. I didn't pursue it further.

He had earned ~~his~~ a niche for himself in the field of haemoglobinopathies both on the national and international scene. He was associated with this field, right from the early discovery of sickling among the Todas of Nilgiris in India in 1951, by Prof. Lehmann and Dr. Marie Culbush, now Marie Cruickshank. He has to his credit many new haemoglobin variants from our country.

The truly remarkable thing about him was, he reached this position of eminence armed only with the most basic High school Qualification — this was ~~his~~ only academic qualification I am unaware of any scientist from India & abroad, who in similar circumstances achieved so much in our times. Later on he was an examiner for M.Sc and Ph.D at many universities in India.

He was a member of the expert panel on Haemoglobinopathy and

. Thalassemia under the International committee on standardisation in Haematology (ICSH) 1977-79.

He represented India on the South-North Round Table on Haemoglobinopathies by invitation, by the Third world Academy of Sciences (TWAS) at Trieste, Italy in 1986.

Given after his retirement from the Cancer Research Institute, Parel in 1982, at the age of 62 (ie after two years extension) he shifted to a new laboratory, (at B.J. wadia children's hospital), organising it from scratch, continuing the work on the forefront of research in his chosen field. This brought the lab international recognition being the only reference lab for haemoglobinopathies in our country recognised by the W.H.O.

He was 76 years young when he passed away just after a hectic visit to Sri Swaminathan's Dept. at Oxford to learn the newer techniques in

prenatal diagnosis, which was to form an important part in a new DNA programme for control of Thalassemia in our country.

I am enclosing xerox copies of some of the tributes received on his passing away to give a glimpse into the personality of this wonderful human being.

I fervently hope this time you wouldn't say No and would go ahead with the idea of bringing out an editorial on him. This would be a fitting tribute to my dearest uncle and a life devoted to science, a source of inspiration to many an upcoming Scientist.

With kind regards,

Yours sincerely,

Krishna

Dr (Mrs) KRISHNAJA, A.P
Molecular Biology & Agri. Div.
Bhabha Atomic Research centre
Bombay - 400 085
India.

Dear Dr. Garfield, It was during my
federal days way back in 1978, that
I first came across "current contents".
Since then I have been an avid
reader of the same. It is with eager-
ness I always look forward to your
editorials and the "citation classics".
That animated anticipation always
remains, although I will be three weeks
behind. I really enjoy reading it
and it indeed is a treasure house
of knowledge put through in a simple
style. I greatly admire your style
of writing.

Over the years, I have read with interest your account of the noted laureates and other prestigious award winners, the skills, broken dreams and about their life, the human side of the story. But none have inspired me so much as the story of my own uncle, who instead of all odds, have carved a niche for himself in the field of science, not only in our country, but in the international scene as well. It was only a matter of chance that he happened to be my uncle. I think in this era nobody can boast of having only a makeshift scientific on paper and still be known for his original contributions in the field of pharmaceuticals. Of course, he had reached the top in the field, where degrees don't matter any more. As J.P.S. Haldane had put it: It is not academic degrees that count, but a persons interest in the subject.

Many yrs after dinner session was devoted to the interesting life history of the boy from he came up and this is as I heard it from him. He used to tell me that one thing that had most influenced him as a young boy was a small booklet in Malayalam (ie our mother tongue) given by his father. The title of the book read "vidhiyodu poruthi-yavar veeralimaakkal". - Translated it means. Brave are the souls who fought against fate. It contained the biographical sketch of some famous men in science. His life-story is a living testimonial to the ideas contained in that book. This indeed must have been the moving spirit behind the lad of 16, who left home after the 40th day ceremony of his father's demise was over. With only a pair of clothes and

a matriculation certificate at 16 the left home telling his mother he will come back, if he succeeds in getting a job. He went to Bombay in search of a job at the Indian Institute where his father was working till his retirement. When he reached 18, he got this job as dak attendant at Indian Institute, Bombay.

In 1952, he left Bombay and joined the Indian Cancer Research Centre, the present Cancer Research Institute at Parel. He had a myocardial infarction in 1965 and the doctors gave him six months time to live. But he continued to work with rigid personal discipline, overcame the physical illness and at 68 he looks a sprightly middle aged man with a walking stick for him and he continues to work at the Research laboratories

for haemoglobinopathies established by him at Wadia Children Hospital Bombay (this is the only W.H.O recognized centre in India).

I am enclosing herewith two Pic-data and some other relevant details to give a glimpse into the study. If you think this study will be an inspiration to many young scientists and if so with an editorial, I will be delighted to provide you with further details.

Thanking you and with kind Regards,

Yours Sincerely,

Krishnaja
(Krishnaja).

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Krishnaji.

The Fourth deSa-Sanzgiri Oration

under auspices of

Dr. Ramesh Sanzgiri Foundation

at Margao-Goa

on 16th September 1984

Kindly return this copy after going through it.

Thalassaemias and Hemoglobins

by

Dr. P. K. Sukumaran

Dr. Arthur deSa

Dr. R. V. Sanzgiri

First quarter of 1981, saw the passing away of two doyens in the field of Pediatrics and Pediatric Surgery. Dr. Arthur Earnest deSa on 4th March and Dr. Raghunandan Vinayak Sanzgiri on 4th April. Both were outstanding men of their times and worked hard to establish their speciality all the way to get recognition. Bombay has always been the "Mecca" of various specialities and it is a matter of pride that many senior teachers in India holding the chairs of Pediatrics today were trained in Bombay under Dr. R. V. Sanzgiri.

Pleasant, full of humour and knowledge on various aspects of life other than professional, both Dr. Arthur deSa and Dr. R. V. Sanzgiri were extremely popular with students, patients and their relatives and medical practitioners at large. B. J. Wadia Children's Hospital indeed brought them close together and they remained best of friends till the end. They were never self-centred and generally wanted the best done for their patients and students. They taught with enthusiasm and students enjoyed their teachings, and these students have indeed become a lasting monument to their vision and industry.

In instituting this deSa-Sanzgiri oration, we are Honouring the memory of these giants in the field of clinical medicine.

Dr. Ramesh Sanzgiri Foundation

Dr. P. K. Sukumaran
HEMATOLOGIST
Bai Jerbai Wadia Hospital For Children
Parel

I consider it a great honour to be invited to give this deSa Sanzgiri Oration this year. I am very thankful to the Foundation and Dr. Ramesh Sanzgiri in particular for affording me this opportunity. Both Drs. deSa and Sanzgiri were known to me for more than two decades and both were luminaries in their own profession, yet humane in thought and action. Dr. Sanzgiri was my well wisher and I always looked to him for valuable guidance, both in my professional career and personal welfare and I received them in plenty. Today's function gives me an opportunity to pay my humble homage to these giants in their own respective fields of speciality and yet thorough human beings. Though not belonging to the medical fraternity this invitation to address this august gathering is a great privilege given to me by the Foundation.

I wish to share some of my thoughts as I look back at the three decades of my work in the field of haemoglobinopathies and I look forward to the scope of management for the control and prevention of the disease in years to come. I have selected the following title for the topic of my talk.

Thalassaemias and Haemoglobins I have known — some stray thoughts.

To embark on anything new and unknown so far, is not an easy task and this was true in my attempt to look for sickle cell haemoglobin in this part of the country. Thalassaemia was already reported in India as early as 1938 from Bengal and later in the early 1940s by K. E. M. Hospital group and J. J. Hospital group in Bombay. To search for sickle cell gene in India was like asking for moon,

because in those days sickle cell anaemia was considered characteristic of Negroes and those with Negro ancestry in other parts of the world. In 1952 when working in Pasteur Institute, Coonoor, Nilgiris, and engaged in the work of serology of syphilis among the Todas of Nilgiris, a request came to the Director of the Institute from ICMR for some laboratory space and assistance for a team from London wanting to look for sickling in the tribes of Nilgiris. Being already familiar with field work, especially among the Todas, I was asked to join them although I never heard of sickling phenomenon, leave alone seeing one under the microscope. The team consisted of Prof. H. Lehmann (then of St. Bartholomew's Hospital) who later on became my benefactor and guide even to this day; and Miss Marie Cutbush (Now Mrs. Marie Crookston) from whom I learned blood grouping technique. Incidentally, first time Coombs reagent was prepared in 1952 with her help and using her serum for immunization of rabbits which we called Australian strain of Coombs reagent.

In response to my request to demonstrate to me the technique for detecting sickling phenomenon my Professor proceeded to do sickling test using sod. metabisulphite, on some staff members of the Institute. It was in this attempt the first case of sickling was detected in one of the laboratory staff of the Institute. This was a great excitment to all three of us and we still remember this day when the first case of sickling was seen. We then continued this work with success in many tribal groups and with non-tribals as controls. This I consider as a land-mark in the study of abnormal haemoglobins in India.

In 1953 I had moved on to Indian Cancer Research Centre (the present Cancer Research Institute) entrusted with the task of working in a team on blood groups at the population level, in Bombay and surrounding areas.

It dawned on me that if sickling was found in the tribals of Nilgiris it could also be found in the tribal population of Western India and I was toying with the idea to start such a study. I remember the day I was admonished by none other than the erstwhile Director of ICRC the late Prof. Khanolkar, who considered looking for sickling in Western India as a futile attempt. He was right in a way as by then mixed feelings were expressed in medical journals about the unexpected findings of sickling in non-Negro population, specially Indians. Even the whole report published in British Medical Journal was questioned as possible example of false positive sickling due to drying phenomenon as they were carried out in the field in tropics.

It was at that time one of my colleagues Mr. G. N. Vyas (Dr. Girish N. Vyas, Ph. D., Prof. of Laboratory Medicine and Director of Blood Bank, University of California, a distinguished scientist and an outstanding worker in the field of hepatitis B virus) was doing his postgraduation thesis on genetic parameters in the tribes of Gujarat. My request to pass on to me the blood samples, after his work, was straightway agreed upon—a benevolent gesture! Not having sod. metabisulphite for use in sickling test was a great limitation in my attempt to study the samples. However, as an alternative, foeces samples emulsifield in saline and clear supernatant was used. One day to my pleasant surprise, I detected the first case of sickling positive sample from a Dhodia tribal. No mention was made to any one till I found more positive samples. Yet I had no courage to inform this finding to my director although I informed the finding to the Head of my Division, Dr. L. D. Sanghvi. One day with all my courage in hand I went to my Director and appraised him of the findings. To my surprise and joy, I got a pat on my back with a remark " well done ".

By this time I had exchanged correspondence with Prof. Lehmann who was by then itching for confirmatory test for the sickling gene found in India. At his suggestion I took a repeat study on the same tribes in Nilgiris with additional test of electrophoresis for confirmation, to which I included the tribal samples from Gujarat. These attempts were successful and the results published and thus buried the contraversy and criticism, established that Indians do have the genes for sickle cell haemoglobin. Later on workers from other parts of the country confirmed our findings. This was a hallmark in my career and I switched on to the study of abnormal haemoglobins and bid goodbye to blood groups.

At one of the clinical meetings at Bai Jerbai Wadia Hospital for Children I had given a talk on the findings of sickling gene in the tribes of Western India when both Dr. Sanzgiri and Dr. S. M. Merchant were present. As an offshoot of this, to my surprise a child 5 years and his father, a doctor and student of Dr. Sanzgiri came to me through Dr. S. M. Merchant. This was for investigation for sickle cell anaemia and the presenting symptoms were splenomegaly, jaundice with history of bouts of fever when complaints of joint pains were present, not responding to conventional treatment. This was our first case of sickle cell-thalassameia with father (Gujarati-Teli) carrying beta thalassaemia trait, mother (Hindu-converted christian) with sickle cell trait. Of the other two siblings (twins), one was thalassaemia trait and the other sickle cell trait. This findings created an uproar and Dr. Sanzgiri came personally to me to discuss, when we decided to find out mother's ancestry which turned out to be Bhil converted, two generations ago, and this solved the problem.

It is now known that Hb S is not the prerogative of the tribals but is seen in many scheduled castes (Mahars,

Gonds, Phul Malis etc. from Maharashtra) and (Mayavanshi, Sorti etc. from Gujarat). All cases of sickle cell anaemia and sickle cell–thalassaemia do not show severity of clinical manifestations which is usually a rule rather than exception. Increased foetal haemoglobin in these cases sometimes show ameliorating effect on severity. It is now known that in the Middle East the sickle cell gene is of different type compared to those found in Blacks in Africa and U. S. This difference was detected by recent methods of gene mapping studies which showed that the Hb S gene is situated on 13 kb (kilo base) fragment of the gene while those in the Middle East and in some limited samples from India are on 17.6 kb using restriction endonuclease Hpa I. Similar studies using on variety of restriction enzymes on samples of Hb S from different parts of this country can be useful to establish heterogeneity, if any, in this sub-continent. This may also give a clue on gene flow if any.

A careful observation by a student of Pediatrics, on a patient with suspected purulant meningitis showing dark blue lips on crying made him suspect methemoglobinemia, and referred to us. On careful questioning it was found that father and three sibling of the baby also had their lips and finger nail beds blue. Our investigations revealed this to be Hb M which on characterization found to be Hb M (Boston).

A case of congenital erythropoietic porphyria together with beta thalassemia in a small Koli child was suspected when urine collected for examination turned brown on keeping. This as a pointer along with clinical findings supported by fluorescent microscopy showing primary fluorescence, confirmed the provisional findings. These are few instances when young doctors with inquisitive and careful observation helped to detect rare cases of clinical importance.

Thalassaemias

Our interest in investigations for thalassaemia started as early as 1952–53 when a popular surgeon was directly interested with his daughter suffering from thalassaemia and maintained on blood transfusion. Thalassaemia was not unknown in Bombay area. Though this was described from Bengal in 1938, cases answering the description of beta-thalassaemia were reported from a group in KEM hospital and another from J. J. hospital. Investigations used for diagnosis were rather limited and we on our part, gradually started laboratory diagnosis for thalassaemia. One strategy we followed was to insist on getting both parents investigated along with the patient to establish homozygosity if present in the child, with heterozygous state in both parents. This helped us a great deal in arriving at a correct diagnosis, either as thalassaemia or its variants. An important observation to note, is that investigations after a blood transfusion showed that endogenous haemoglobin is depreseed and it became impossible to detect accurately the amounts of Hbs A, F and A2 even if estimations were carried out at long intervals after transfusion.

At this stage it may be useful to recapitulate that normal human haemoglobin is heterogeneous and abnormalities of haemoglobins and thalassaemia mainly lies in the globin part of the haemoglobin molecule. Normal adult haemoglobin consists of a pair of alpha (α) chains and a pair of non-alpha chains and constitute three types of haemoglobins. They are Hb A with a pair of alpha (α) and a pair of beta (β) chains: Hb F alpha2 ($\alpha 2$) and gamma2 ($\gamma 2$) and Hb A2 alpha2 ($\alpha 2$) and delta2 ($\delta 2$). Hb A is found to be about 97% ; Hb A2 about 2.5% and Hb F about 0.5% of the total haemoglobin. It can be seen that alpha chain can reflect on all the three

fractions and any abnormality in this chain reflect on all the three types of haemoglobins. There are embryonic counterpart of these globin chains. Genes for Alpha and its precurser are on chromosome 16 and those for beta, gamma and delta and their precursers on chromosome 11.

For diagnosis of thalassaemia major (Cooley's anaemia), thalassaemia trait and their variants, investigations in our laboratory include detailed study of hemogram now using electronic counter; osmotic fragility of red cells, reticulocyte count, estimation of alkali-resistant haemoglobin and haemoglobin A2 quantitation, besides careful screening of peripheral blood smear. Electrophoresis of haemoglobin is routinely done on all samples. Blood picture compatible with microcytic hypochromia supported by decreased osmotic fragility of red cells, marginal increase in reticulocyte count and increased levels of Hb A2 are findings suggestive of beta thalassaemia in the absence of any electrophoretically detectable abnormal haemoglobin. MCV and MCH are reduced while MCHC may be normal or near normal. Iron deficiency showing similar blood picture will show low MCHC and decreased levels of Hb A2 besides serum iron studies confirm the findings. In thalassaemia major (Cooley's anaemia) marked anaemia with low levels of haemoglobin, low MCV, MCH and near normal MCHC, recticulocytosis and markedly microcytic hypochromia with fair amounts of nucleated red cells seen in blood smear are the findings. Increased level of Hb F (alkali-resistant Hb) usually above 30% in fresh cases of beta°-thalassaemia. Osmotic fragility is usually decreased unless microspherocytes are present in fair numbers.

As a screening test for Hb F we do a simple qualitative test which we recommend to Pediatric students. This can easily be done in the clinical side room. As

increased Hb F is the main feature of beta-thalassaemia major, this could be useful. Take 5 ml. of dist. water in a test tube and few drops of patient's blood to have a fairly dark colour of haemolysate. Add 1 ml. of 1% NaOH solution and watch for the change of colour by noting the time of start of reaction. If the colour changes to greenish brown from original bright red **within 1 minute,** indicate normal levels and there is increase level if the colour (red) persists for more than 10 minutes the level of Hb could be more than 40% mostly seen in fresh cases of betathalassaemia. This could be used even as bedside test on finger-prick blood to find out whether Hb F is markedly increased.

Beta-thalassaemia interacting with beta-chain variant abnormal haemoglobins (Hbs S, D, E.) are not uncommonly seen. They show electrophoretic mobility pattern simulating homozygous condition. Of these Hb D-beta thalassaemia though showing only Hb D without Hb A are normal clinically. This findng helped us to contradict a report of Hb D disease reported earlier in a Sikh soldier which was later confirmed as Hb D-thalassaemia.

Haemoglobin Q India (alpha 64 Asp-His)

Haemoglobin Q, an alpha chain variant with beta thalassaemia a rare combination was found in a few families of Sindhi community in Bombay. This abnormal haemoglobin (Hb Q) with amino acid substitution at a hitherto not reported position has become a new haemoglobin variant. This double heterozygous condition had haematological features of beta-thalassaemia trait with no clinical manifestation. This haemoglobin variant could be easily detected by electrophoresis even in the presence of beta-thalassaemia being a non-interacting alpha chain type.

As said earlier, the carrier of this haemoglobin showed variant types both in foetal and Hb A_2 an easy way of detecting an alpha type of haemoglobin.

Haemoglobin J Thalassaemia

This was detected in the mother of a child with beta thalassaemia major and belonged to Gujarati-Lohana community, father thalassaemia trait. Mother was clinically and haematologically like thalassaemia carrier. This was an alpha-chain variant. When reported in 1958 this was a very rare combination.

Hereditary Persistence of Fetal Heamoglobin (HPFH)

Foetal haemoglobin starts disappearing slowly after birth and come down to normal level (0.5 to 1%) after six months of age. It is found increased marginally in beta thalassaemia trait and higher values are seen in disease conditions such as beta thalassaemia major; sickle cell disease: sickle cell thalassaemia; Hb E thalassaemia etc. Foetal haemoglobin is found increased in some forms of haemopoietic malignancies also. In 1953 the then senior Physician late Dr. R. V. Sathe and haematologist late Dr. J. G. Parekh had seen a Christian boy 18 years with jaundice, splenomegaly, anaemia and other features of thalassaemia which was then referred to us. We found that haematological features were in favour of thalassaemia with increased foetal haemoglobin (46%); mother a beta thalassaemia carrier and father was absolutely normal haematologically. But he showed increased foetal haemoglobin (28%) with no other abnormal haemoglobin on electrophoresis. Detailed family studies showed that this increased fetal haemoglobin condition was inherited in three generation, father, daughter and grand daughter. A similar condition like that of the proband was seen in his one sister with foetal haemoglobin increased (64%) with thalassemic features both clinically

and haematologically. Other two sibings showed beta-thalassaemia trait. No such report of cases with foetal haemoglobin found raised to more than 20% with absolutely normal haematological picture was found till then in literature. Hb F was evenly distributed in the cells as found by elution staining method. We suspected this to be a different entity. About this time we had two world famous haematologists visiting us. They were Prof. Maxwell Wintrobe and Prof. Damashek of U. S. A. We presented the boy (propositus) to each of them on different occasions, with results of blood examination and bone marrow findings. The verdict was that this was nothing new but a frank case of thalassaemia intermedius. As for the family, the opinion was that they were thalassaemia traits but foetal haemoglobin increase was brushed aside as technical error. Opinions were almost identical from both these haematologists. This poured cold water on our enthusiasm of having dctected a new entity and needless to say that we could not publish the findings. The most disappointing part of the story was that we did not have the courage to publish our findings. as a new entity, after the verdicts from the experts, and later in 1957 Went and McIvor from Jamaica reported Sickle cell trait with hereditary persistence of foetal haemoglobin. This was a great blow for us having lost the priority. Later on many cases of such double heterozygous conditions seen were by us and others in Bombay. Such situations can arise in anybody's professional career. My advice to younger scientists is that if they have the courage of conviction that they are right, they should not be led away by others' opinion. We learned this at great cost.

It is now known that HPFH conditions are of many types of which the ones that show Hb F more than 20% (20–30%) with pancellular distribution of Hb F is classified

as Negro type. Our cases and most of the ones reported from other parts of India are of this type. But the Negro-type with beta°-thalassaemia do not show such clinical manifestations and haematological severity in such entities seen outside India. This made us suspect that our HPFH may still be different. With this in view I carried DNA samples extracted from our HPFH samples to do gene mapping studies and compare them with the Negro types found in the USA, by me and my colleagues at Cell and Molecular Laboratory of the Medical College of Georgia, at Augusta. It is interesting to note that the Indian HPFH showed much larger deletion including the delta and beta genes when compared to two groups of Negro samples. This perhaps explains the clinical and haemaological severity seen in our cases of HPFH with beta° thalassaemia.

Gamma (ϒ) thalassaemia

A cord blood survey for the occurance of different levels of G-gamma (Gϒ) chains and A-gamma (Aϒ) chains in foetal haemoglobins were carried out in Bombay. It may be said that normally there are two types of gamma chains in foetal haemoglobin, one with glycine (G) at 136 position and the other with alanine (A) at the same position. Study of the proportion of these two show the ratio changes from 7:3 at birth to 4:6 at 6 months and above, while absence will indicate gamma-thalassaemia of either of the varieties. In one of the samples of cord bloods we found low levels of foetal haemoglobin at birth and the disappearance was faster than normal with increasing age. This child was otherwise normal born of consanguinous parents. Suspecting this to be a possible case of gamma-thalassaemia after all the initial studies, here we wanted to have DNA analysis done. Blood sample collected, processed and taken to Augusta

and with my colleagues up there we could have a gene mapping done. To our surprise this turned out to be the first example of a deletion of one of the two gamma chains genes – an example of gamma–thalassaemia.

Now let me look at the future. What is it that we can offer to these unfortunate families with thalassaemia and sickle cell anaemia children? Molecular biologists are advancing at rapid speed with the hope of finding something to offer to such patients. It is a multifaceted programme that is going to help in this situation. It is well known that incidence of thalassaemia varies in different ethnic groups ranging from anywhere between 1.8 to 17.5%. This information is fragmentary as the data available included only very small population and that too in limited areas where facilities are available. Management or treatment of the disease is not my cup of tea, but by keeping abreast of literature on the progress made in other countries and having had first hand knowledge of the working of such clinics engaged in the management of thalassaemia I wish to share some information.

At present repeated blood transfusion is the only way of prolonging the life of these patients. Frequency of transfusion differes from case to case and as the case advances. Some have advocated neocyte (reticulocyte rich) blood transfusion. It was found this blood lasts longer in the system when compared to whole blood transfusion. But to get one unit of blood with neocyte rich red cells, one has to atleast process three bottles of blood. In some laboratories using a cell separator they are able to collect blood, separate reticulocyte–rich red cells and put back the rest into the donor. In any case repeated blood transfusion poses problems of iron overload and associated clinical manifestations. Hyper transfusion

by maintaining haemo level between 11 and 12 gms.% has its benefit. The child is active, growth retardation is less. This problem of obtaining sufficient blood donors, severe iron overload and to remove excess of iron, chealating agents are used. Desferrioximine is the commonly used chealating agent. This is not available in our market and is frightfully expensive. In clinics abroad subcutaneaous injection by means of pump (syringe driver) which continuously injects small quantities for long periods is preferred in place of intramuscular injections in higher dose for few days at a time. It is believed that in the latter, continuous level of the drug is not maintained and always a doctor's service is necessary. Pump can be carried on the body using a belt and is usually worn while in bed, though it can be used even while walking. The dose used is less and reduces the cost of treatment and at the same time is more effective.

In our experience a child diagnosed in 1955 lived with blood transfusion (at monthly intervals) along with Desferal given intramuscularly and sparingly due to non-availability, for 23 years and died due to complications of iron overload. Hypertransfusion regime could not be used as Desferal was not available in enough quantities. Though we have problems of blood donors experiences from abroad on the successful management of Cooley's anaemia cases is heartening.

Bone Marrow Transplant

First successful transplant was performed in USA with UK and Italy following. Marrow transplant is still a highly sophisticated technology and the procedure is very expensive (estimated cost in UK is between £ 2000–4000) per case. Some argue that even this is less than the cumulative cost of treating a thalassaemia patient

with blood transfusion in a developed country, for ten years. If transplant could be carried out routinely and accepted safely, it should become the treatment of choice. Graft rejection and fatal complications due to marrow suppression or graft versus host disease are the biggest problems that need consideration. Basic requirements are the child as young as possible with a fully compatible donor (usually a sibling) who may or may not be a thalassaemia trait, and child should have very few blood transfusions. Most still hesitate as the technology is still in its infancy.

Fetal Switch

It is thought that homozygous HPFH cases with 100% foetal haemoglobin live normally although with mild polycythemia, so switching on to foetal haemoglobin production could be beneficial. 5-azacytidine is infused into a patient with thalassaemia major for one week reactivated some gamma chain synthesis and caused transient rise in Hb F during the next week. It was soon found that this powerful drug has severe potential side effects and also said to be carcinogenic.

Antenatal Diagnosis

Considering all the possibilities of keeping the affected child alive (financial, social and emotional etc.) one has many problems to face. One is to offer best treatment possible, prolong life and hope for a day when tangible method of a permanent cure is available (genetic surgery). Another way is by geneting counselling prospective of which we talked about and retrospective by which pick up the carrier state of the disease by population survey and educate them of the possible dangers of having a spouse with a carrier state of the disease, leaving the choice to them to make a decision on their own and thus help prevention.

Antenatal diagnosis is very practical way of preventing any affected child being born and avoid all connected problems. But this has to be done after educating the couple in advance of the pros and cons of the procedure. Best gestational age for fetal sampling is 17-19 weeks. Now methods of sampling is easy and safe, in the trained hands. This procedure is followed in many places abroad. After finding the sample being proper and adequate, synthesis studies using ^{3}H-lucine incorporation, incubation, fractionation of the peptide chains, rate of synthesis of different globin chains can be detected. Finding of the absence of beta globin chain would indicate that the embryo in question would develop into a thalassaemia homozygote if both parents are found to carriers of beta°-thalassaemia trait. In such a situation it would be advisable to advocate abortion. Even for these procedures properly equipped laboratory with trained personal and trained gynacologist for fetal blood sampling is a pre-requisite, and expensive. But it is hoped that such facilities would be available soon in this country and the cost brought down to a level that it would be available to the needy who can afford it. If the governmental agencies are involved perhaps this facilities may be available free.

Efforts are being made to have an antenatal set-up at the Bai Jerbai Wadia Hospital for Children where the necessary expertise are available. Perhaps when the equipments, which are already ordered, arrive, a start will be made in this direction. There exist some failures which have now brought to the minimum in this procedure and they are reactions of the mother to such manipulation and consequent rejection when not wanted, misdiagnosis and inadequate sampling. Most of these are taken care of in the hands of well experienced persons.

Fetal Diagnosis by DNA Analysis

This involves collection of trophoblasts with biopsy catheter. DNA can be isolated from the trophoblasts collected from the chorionic villi by suction, and for this procedure fetal blood is not required. Foetus is not touched and complications are much less. Restriction endonucleases are enzymes which cut DNA at specific sites giving a mixture of fragments of different sizes which can be separated by electrophoresis. With blotting technique the separated DNA fragments can be transferred on to nitrocellulose filter and the position of the particular globingenes displayed by autoradiography after hybridization labelled genemic DNA probe specific for the gene which is being looked for. This method is good for sickle cell anaemia but not thalassaemia.

In non-alpha-globin cluster, these are restriction enzyme sites which are polymorphic i.e. may be present or absent on an individual basis so there may be individual variation in the length of the DNA fragment obtained by a particular enzyme. This restriction fragment length polymrphysm (RFLP) is inherited in a simple mendelian manner and can be used as linkage markers to define particular haplotype which carry beta thalassaemia mutation. If the heterozygous parents and an existing affected or non-affected child or lateral relatives of parents, are heterozygotes for one or more RFLP, it may be possible to determine a link between RFLP and these mutations. If the subsequent foetus is homozygous for the haplotype consisting of RFLP which is linked with thalassaemia mutation it must also be homozygous, for the disorder. If it is heterozygous for the particular RFLP homozygous beta-thalassaemia is excluded. DNA is prepared from peripheral blood of parents and investigated. This method offers great hopes for the future.

1981

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March 25, 1992

Dr. A. P. Krishnaja
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Dear Dr. Krishnaja:

Your letter was received here in January and I apoligise for the delay in responding.

I have made a copy of the paper on "Thalassaemias and Hemoglobins" by Dr. P.K. Sukumaran and am returning the original, as requested by you. I wish you hadn't sent me the original, but let's hope we can depend on the postal service to get it back to you safely.

Thanks for sending me all this information on Dr. Sukumaran. In the weeks to come I will be discussing this with my colleagues to decide how we can use it. Please be patient.

Sincerely yours,

Eugene Garfield

EG/MM

cc P. Ryan

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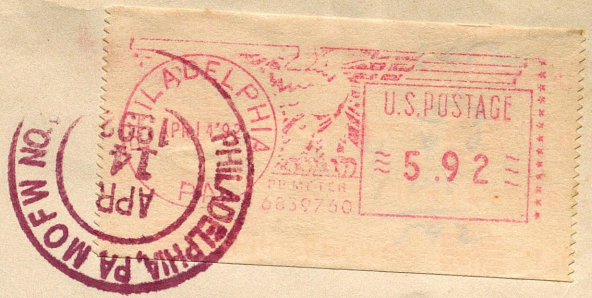

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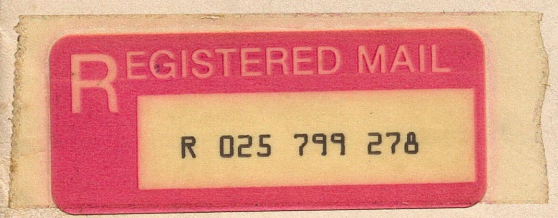


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