THALASSAEMIA:

The Past, Present and Future

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A Background

Thalassaemia or Mediterranean Anaemia is a very common inherited condition affecting haemoglobin production. Haemoglobin is a vitally important molecule in red blood cells. Haemoglobin consists of two different protein chains: alpha (α) and beta (β). If either of these chains do not have the correct structure or are not produced in the correct quantity, the red blood cells may not form correctly and fail to carry sufficient oxygen through our bloodstream. This results in severe anaemia that must be treated with regular blood transfusions every 3-4 weeks. Blood transfusions usually start in early childhood and last a lifetime.

Unfortunately, lifelong therapy of transfusions is also life-threatening because of the build up of toxic levels of iron, which invariably lead to problems with the liver, heart and other organs. Treatment to remove the excess iron from the body is vital from an early age to prevent life-threatening complications.

It is a common misconception that thalassaemia is largely confined to people of Mediterranean background but in fact thalassaemia is a common condition seen worldwide. Distribution studies indicate that thalassaemia occurs at a high frequency stretching from North Africa, Mediterranean region, through to the Middle East, the Indian subcontinent, to South and East Asia.

Surprisingly, it has been estimated that about 7% of the world's population are carriers of various types of thalassaemia or haemoglobinopathies. This high frequency is almost certainly the result of human adaptation and natural selection to malaria infections. It is well documented that malaria had a severe effect in terms of both morbidity and mortality on human populations in antiquity. It killed or debilitated people to the extent that it altered the structure of human populations as well as changing human settlements patterns and influencing the natural agricultural systems.

Fortunately for carriers of thalassaemia, in particular young children, they were naturally protected against lethal form of malaria. Given that children

with this trait can survive following an infection, they could then pass their natural resistance -the gene for thalassaemia - to their children and so on.

Over thousands of years, it is not a surprise to see how this trait became very common in regions where malaria is or was once widespread. A number of epidemiological studies from the sixties seventies indicated that about 15% of both Greeks and Cypriots are carriers of thalassaemia. These are the highest frequencies of thalassaemia genes found today in any Caucasian population, posing a major challenge in the rapid identification and counseling. Being a carrier of thalassaemia has little if any impact on a person's health. The most serious cases arise when two carriers of thalassaemia have children. Children born to two parents with the thalassaemia trait have a one in four chance of suffering from thalassaemia major (life-threatening anaemia).

Many Greek Australians carry the gene for thalassaemia but most are not aware until they have an affected child. On the basis of carrier frequency, about 2% of all Greek Australians couples were expected to be both carriers, leading to an estimated 1000 couples of child-bearing age most likely to have an affected child.

It has been estimated 300,000 severely affected patients are born each year worldwide. Sadly, many of these children are born in economically less developed countries with inadequate infrastructure to support them. With previous and current migration trends from endemic regions thalassaemia is being seen with increasing frequency in many parts of the world, including Australia.

There are approximately 500 thalassaemia patients in Australia, requiring regular blood transfusions every 3-4 weeks. Not surprisingly over 60% of adult patients are Greek, Cypriot or Italian. However, it is not anymore a condition that affects people of Mediterranean background. Children from recent refugees represent the largest number of thalassaemia patients. Alarmingly, international experts and public health organizations from less developed countries have expressed concern over the increasing rate of thalassaemia patients, warning that thalassaemia will soon become as devastating as AIDS. To give an example, in India alone, 10,000 patients are born each year. The same is also true for Pakistan, Bangladesh, Thailand and Indonesia.

In Cairo, the Head Clinician Dr El-Beshlawy at the Paediatric Hospital (similar in size to the Royal Children's Hospital in Melbourne) cares for over 7,000 patients. An army of 40 doctors and nurses work to keep these patients alive!

In Australia, the annual cost of providing the necessary level of care has been estimated to be as much as A$120,000 per patient, which equates to A$60 million dollars per year for 500 patients. Greece, a country of only 11.3 million has 3,500 patients, while Cyprus, a population of only 800,000, has 800 patients. You can quickly see how caring for thalassaemia patients could very quickly deplete the health care budget of any developed or developing country.

Introduction of the mandatory premarital certificate

Countries such as Cyprus and Greece were amongst the first to establish successful national screening programs. One of the most successful screening campaigns to date, a mandatory premarital certificate was initiated by Cyprus. The over all effect of this campaign was so successful that it is now modelled by many countries worldwide. It is interesting to examine the course of the events, which unfolded resulting in the introduction of the mandatory premarital certificate.

In Cyprus in the early 1970s about 80 babies with thalassaemia were being born every year, and the physicians who were treating these babies began to worry about how their economically struggling government was going to afford to treat all the patients. If Cyprus offered the best available treatment to the current patient population, each patient would likely live into middle age.

In the absence of a thalassaemia prevention program, it was estimated that the prevalence of β-thalassaemia would go from 1:1000 to 1:138 over the next 50 years, creating an increase of 400% in the demand for blood and 700% in the cost of treatment. It was predicted that the needs of its thalassaemic patients would completely overwhelm not just the available blood supplies but also the entire budget of the Ministry of Health, compromising the quality of care not only for the existing thalassaemia patients but for other patient groups as well.

Voluntary population screening was thus considered, but proper counseling of all persons examined presented major difficulties due to the unavailability of trained genetic counselors. Preliminary voluntary screening projects of high school students and army recruits proved very ineffective, since the

candidates did not know enough to make an informed choice about their testing. By the late 1970s, prenatal diagnosis of blood had become possible and by 1980 there was a reduction of more than 50% of the number of thalassaemia patients born. This was partly due to a significant number of couples going to London for prenatal diagnosis but also due to the postponement of pregnancies by many couples that already has a thalassaemic child.

Following the introduction of prenatal diagnosis in Cyprus in 1981, the uptake was almost complete. As a result, the number of babies born with thalassemia began to fall, slowly at first and then more rapidly. By 1982, only eight thalassaemic babies were born 90% fewer than what was originally predicted.

Nonetheless, several of the thalassaemia specialists remained unsatisfied because the number of babies born with the thalassaemia each year had not yet fallen to zero, which is where they wanted it to be. This was often the result of failure to inform the couple sufficiently by some doctors or of laboratory errors when testing was performed by private laboratories since there was no adequate quality control system in place.

By the early 1980's, the Cypriot Antianaemic Society began to lobby the government to mandate screening, but they were regularly refused, as no legislator would vote for it, fearing condemnation by the Orthodox Church. As a consequence, the physicians and parents requested a meeting with Archbishop Chrysostomos (then the head of Church) and the bishops who consult with him on Church policy. They presented three arguments in favor of mandating a premarital certificate.

- First, reduce suffering, which a premarital screening program would unquestionably do, since it would reduce, to zero with any luck, the number of babies born with β-thalassaemia.

- Second, as an opponent of abortion, he needed to understand that premarital screening would, also unquestionably, lower the abortion rate amongst Greek Cypriots, since most women in heterozygote marriages who were not using prenatal diagnosis were terminating all their pregnancies subsequent to the birth of a thalassaemic child.

- Finally, the patient advocates argued, as the head of a community that was diminishing in size (because of emigration and a lowering birth

rate) the Archbishop ought to do all he could to encourage men and women in heterozygote marriages (roughly 1 in 49 Cypriot couples were both carriers) to have as many children as possible.

In 1983, as a consequence of unanimous support from physicians, parents, politicians, and the head of the Church, Archbishop Chrysostomos, announced that, henceforth, priests of the Cypriot Orthodox Church could request a certificate from all Greek Cypriot couples wanting to be married in the Church. The certificate, which was soon formalized, was to be issued by the Cyprus Thalassaemia Center in Nicosia; it attested (and still does) to the fact that an individual had been both screened and counseled at the Center.

The certificate of the laboratory results was in no way compromised, since the certificate presented to the Church authorities only state that the bearer of the certificate was examined and properly advised for thalassaemia. The actual diagnosis was given to individuals in the form of an identity card for their private use.

The outcome of the pre-marital certificate allowed the couple time to decide on the best reproductive option. As a consequence, the number of babies born with thalassaemia dramatically dropped from 80 per year to nearly zero. The success of this prevention program quickly gained international recognition and is now modelled by several countries around the world.

In Australia, a mandatary pre-marital certificate would be very difficult to implement due to cultural differences and also on the grounds of ethical and legal issues. Instead prenatal testing for thalassaemia is routinely performed to determine whether or not the pregnancy is affected by thalassaemia.

All women have a full blood examination early in pregnancy and those who are iron loaded with a low mean cell volume are tested for thalassaemia carrier status. The outcome is that it is now uncommon for babies to be born with thalassaemia.

Ideally, screening would be performed before conception as is performed in Cyprus with the main emphasis being on prevention. However, for the many at risk couples routine prenatal screening may be a difficult time to decide on the most appropriate reproductive option.

Reproductive options

Advances in reproductive technology have opened new opportunities to assist

carriers who do not want to risk giving birth to a child with thalassaemia.

Preimplantation genetic diagnosis (PGD), used in conjunction with in vitro fertilisation, may enable parents who carry the trait to give birth to healthy babies. It involves the removal of one to two cells from eight-cell embryos after in vitro fertilization, the diagnosis and detection of thalassaemia before being implanted into the mother, allowing only healthy embryos to be selected.

There are now over 130 disease genes including those with a late onset have been identified and the conditions averted in children. Risks of abortion have been reduced, and the incidence of live births rose after PGD. It is also now possible to select embryos with human leukocyte antigens (HLA) identical to those of a sick older sibling, and then use stem cells from cord blood at birth to supply the necessary therapy and prevent the devastating immune complications of graft rejection. This form of treatment has alleviated the inherited disorder in many recipients.

Therapeutic Strategies

For the hundreds of thousands of patients born each year, bone marrow or cord blood transplantation is the only cure available but it depends on finding a compatible donor, which is often not possible or may take years to find. One exciting possibility is the use of gene therapy to treat thalassaemia. This procedure relies on the use of a genetically modified virus to introduce the corrected gene into patient's stem cells. Gene therapy has clearly been demonstrated to be effective in a limited number of clinical trials, but despite positive results, there are concerns regarding the safety of this procedure.

While our research group at the Murdoch Childrens Research Institute and other gene therapy laboratories all over the world are currently addressing these safety concerns, it is clear that it is necessary to also broaden research into other strategies that do not pose any risks.

My research group is currently searching to find a more viable alternative. We all express fetal haemoglobin during early fetal life, but just after we are born, the production of this protein switches off. Interestingly, some thalassaemia patients inherit a defect in the "switch" resulting in continued fetal haemoglobin production through to adult life and free from thalassaemia related symptoms. If we can switch "on" the naturally dormant fetal haemoglobin gene we have a means of therapy. The challenge now is to

discover the trigger, but without a clear understanding of the natural mechanisms that controls this “switch” it is virtually impossible to focus on any molecular target. Our research group has produced a number of unique biological tools that we have used to narrow down our search and have now identified some new pharmacological agents, which look promising. However, without further tests it will be premature to say whether these agents can be used therapeutically.

Another exiting new prospect for potential therapy first requires a basic understanding of what contributes to thalassaemia, which is α- to β- globin chain imbalance. The degree of correction is correlated closely with the degree to which globin chain balance has been restored. Hence, alterations in α-globin chain synthesis have considerable effects on β-thalassaemia and can confer transfusion independence. Given that excess production of α-globin leads to widespread detrimental effects in β-thalassaemia, it logically follows that reduction of α-globin synthesis improves the β-thalassaemic phenotype.

Most therapeutic studies have focused on restoring expression of γ- globin (fetal) or β-globin (adult) while very few have attempted to reduce α-globin. Our group recently investigated the feasibility of utilizing RNA interference (RNAi)-mediate reduction of α-globin mRNA. RNAi is a highly conserved naturally occurring mechanism of gene suppression and is mediated by small double stranded RNA molecules termed small interfering RNA (siRNA).

We recently explored the use of RNAi to reduce the expression of α-globin. By decreasing α-globin synthesis we noted a phenotypic change in thalassaemic cells, decreasing reactive oxygen species production to normal levels, thus correcting one of the major disease indicators. This novel strategy raises the possibility of targeting α-globin as a potential therapy for β-thalassemia.

Given that one of the major issues with current gene therapy vectors is achieving high levels of expression we hypothesise that α-globin-specific RNAi sequences can be used synergistically to enhance β- or γ-globin gene therapy to achieve balanced α:β or α:γ globin ratio to non-thalassaemic levels. The RNAi field is a rapidly evolving and a variety of different chemistries have been shown to improve its efficacy. It is likely that further advances in efficacy and delivery will continue to emerge over the next few years and we aim to be in a position to capitalize on these improved strategies and to rapidly integrate these developments into our investigation.

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