B-TALASSEMA: THE ANAEMIA COMING FROM THE SEA
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Introduction
Beta thalassemia is a severe haematological disorder, caused by a genetic mutation. Nowadays, medical treatment and prenatal diagnosis allow to prevent the spread of the disease, but in the past it was largely distributed, mostly in people coming from the Mediterranean region. This fact led to the traditional conviction of the Mediterranean origin of β-thalassemia, the reason why it is also called “Mediterranean anemia”.

Really, its origin is still controversial, but its distribution shows its probably origin and its spread from Greek population especially as far as Italy is concerned and it also shows interlinking relation with the malarial infection (Iandola 1).

The disease results in a severe anaemia that prevents to survive, and in peculiar bone modification which can be detected in the osteological specimens coming from archaeological sites.

Anyway, a certain diagnosis cannot be made only by a macroscopic examination of the material, but there is a need of sophisticated techniques, that allow to discriminate between the modifications that are unspecific and those that are typical of thalassemia (Schulz 2003).

What is Thalassemia?
Thalassemia is a genetic disorder that involves the decreased and defective production of hemoglobin. Hemoglobin is the molecule found in red blood cells (RBCs) necessary to transport of gases, and to give red pigment to RBCs (eMedicine 2007).

Normal hemoglobin is a tetramer made up of two pairs of globin chains (which can be α, β, γ or δ chains) attached to an heme, containing a core of Iron (Figs 1-2). Different combinations of different globin chains produce different types of haemoglobin. For example, haemoglobin of a new born child is only type F (fetal), made of 2 α and 2 γ chains, whereas the normal adult haemoglobin is 95% of type A (made of 2 α and 2 β chains), 3% of type A2 (2 α and 2 δ chains), 1% or less of type F (Badens et al. 2000, Hillard et al. 1996).

Thalassemia disease originates from a defect in the gene or in the sequence close to the gene that settles the synthesis of the different globin chains2, that leads to defective or totally absent production of the correspondent globin chain. This results in an imbalance between the different chains that form the haemoglobin, causing the clinical features of the disease: in fact, for normal functioning of RBCs it is necessary an equal production of α and non-α (β, γ or δ) globin. In adults, the most common imbalance is between α and β chains; that's because the greater part of adult haemoglobin is type A. Really, it can be possible to have also γ or δ thalassemia, but the amount of haemoglobin made up of such chains in adults is too little to develop any symptom (Badens et al. 2000).

1 www.demarchi.org/thalassemia.htm
2 β, γ, δ genes control the synthesis of β, γ, δ chains and are localized on chromosome 11, whereas α gene, found on chromosome 16, settles the synthesis for α chain (Hillard and Barkou 1996).
In β-thalassemia, in particular, there is a decreased or totally lack of β-globin synthesis, which results in an excess of free α chains, that accumulate and precipitate damaging both erythroid precursors in the bone marrow and circulating RBCs. In fact, red blood cells are made in the red bone marrow; here, stem cells multiply and differentiate into the different blood components, such as “erythrocytes”, “leukocytes” (which fight against infections) and “platelets” (which are responsible for healing wounds; Fig. 3). The destruction of erythroid precursors caused by the excedent α chains results an in ineffective erythropoiesis; RBCs are smaller than normal (microcytosis) and have few levels of haemoglobin (hypocromatosis; Figs 4-5), so they are not able to carry enough oxygen through the body (Childrencentralcal 2006, eMedicine).

The blood production is also under spleen control\(^3\); in β-thalassemia, the spleen receives less oxygen and secretes more erythropoietin, a hormone which stimulates RBCs production into the bone marrow to have more circulating haemoglobin so as to have enough oxygen carried away. But the abnormal RBCs are not able to face this request and, over time, this mechanism leads to proliferation of the bone marrow. Moreover, the surviving cells that arrive in the peripheral blood are subjected to hemolysis caused by the excess of free α chains that can’t tie to some others: both hemolysis and ineffective erythropoiesis cause anaemia in a person with β-thalassemia (Childrencentralcal 2006).

\(^3\) In fact, the spleen acts as a reservoir of RBCs (Wikipedia).
Thalassemia is not infectious, it can be only inherited, transmitted from parents to sons in an autosomal recessive pattern. This means that if both parents carry a thalassemia gene, there’s 25% chance with each pregnancy to have a child without genes of thalassemia (a totally healthy child with normal hemoglobin), 50% chance to have a child with only one gene affected, and 25% chance to have an affected child, carrying both genes for thalassemia (Figs 6-7). Therefore, it is necessary to inherit abnormal genes from both parents to develop the full-blown disease (Arzisanrovigo, Cooleysanaemia).

If only one parent passes the thalassemia gene to a child, then the child is said to have the Thalassemia trait (Thalassemia Minor), the heterozygous form of the disease. Thalassemia trait can be done both for α and β gene, and, in both cases, will not develop into the full-blown disease; usually, it produces no or few symptoms (e.g. only a mild microcytic and hypocromic anemia) and no medical treatment is necessary (Wikipedia).

The homozygous form for β gene mutation is called Thalassemia Major (Mediterranean Anaemia or Cooley’s Anaemia). It is a really severe microcytic and hypochromic anemia that, if not treated in time results in death very early, usually before the age of 20. The symptoms usually begin within 3-6 months at birth, when haemoglobin F starts changing into haemoglobin A, becoming evident during the second part of the 1st year of life. Besides anaemia, they include jaundice, asthma, chronic fatigue and listlessness, growth problems, increased susceptibility to infection, hormone problems (e.g. delayed or absent puberty, diabetes, thyroid problems), heart failure, shortness of breath, liver problems, gallstones, extramedullary hemopoiesis which can lead also to neuropathy or paralysis due to the compression of the spinal or peripheral nerves by extramedullary hematopoietic masses⁴, hypersplenism due to spleen overload, and, the most important for a paleopathologist, bone marrow hyperplasia (Hollnstein 2006 in MassachussetsGeneral Hospital, Zafeiriou et al. 2006).

In fact, the most severe changes caused by β-thalassemia appear in bones, and are totally due to the increased spatial demands for the hyperplastic erythropoietic marrow. In keeping with the general distribution of the red bone marrow⁵, the entire skeleton of the child is affected more or less uniformly, whereas in adult, characteristic bone changes remain only in areas of permanent hematopoietic activity. The location of these signs follows the process of conversion of red bone marrow into fatty marrow; so it starts at the extremities to involve during adolescence the spine and the proximal epiphysis of the limbs (Hillard and Berkow 1996, Kalifa and Mergouz 2004).

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⁴ Economou et al. 2006.
⁵ The hematopoietic one.
The most severe changes affect the skull, which is characterised by a marked porotic hyperostosis; the porosis usually starts in the upper portion of the frontal bone (which often shows thickening and frontal bossing) (figs. 8, 9), and then extends to parietals (Fig.10) in a symmetric way. The cranial vault is characterized by a marked expansion of the diploë that becomes visible through the external bone surface, which is progressively eroded and destroyed (Fig. 12). The trabeculae of the diploë are reduced in number and are accompanied by thickening and radial rearrangement of the remaining, which creates the characteristic hair-on-end appearance (one of the most important marks of β-thalassemia; Fig. 11). The inner surface is usually only slightly affected in a really advanced stage of the disease. In this situation, the porosis may extend also to other cranial bones, such as sphenoid, mastoids and cheekbones (Fig. 12). Such modifications appear during childhood and growth phase, when the greatest amount of iron is required by the organism, and then can heal. Thus, the modules of red bone marrow toward the external bone surface can be remodelled secondarily, and can be seen as radial striations at the edges of the porotic areas; this secondary change in the bone structure of the vault is an evidence of healing processes (Fig.13; Aufderheide 1998, Ortner 2003).
Characteristic changes appear also in facial bones as a development of frontal bossing and a thickening and roughening of maxilla and zygoma that lead to a mongoloid appearance characterized by prominent cheekbones and hypertelorism. The involvement of maxilla and mandible leads to disorderly eruption of the teeth and malocclusion of the jaws and creates the characteristic rodent aspect of thalassemic children (Hillard and Berkow 1996, Kalifa and Mergouz 2004).

The axial skeleton shows widening and reticulation of the ribs, especially in the posterior portion, due to reduction of the total mass of trabecular and cortical bone and arrangement of the remaining trabeculae along stress lines, and porosis of the vertebrae, with decreased height, increased width and cupping of the endplates, that lead to the typical fish-vertebrae aspect. Compression fractures, due to the marked osteoporosis, may occur in the lower dorsal and lumbar areas (Aufderheide 1998, Ortner 2003).

Long bones usually show a marked widening of the medullar cavity accompanied by the thinning of the cortex\(^6\), that may become reticulate and normally has marked enlarged vascular foramina containing hyperplastic marrow, a marked porosity and a typical flask-shaped deformity of the metaphyseal area of tubular bones ("Erlenmeyer flask deformity"; Fig. 15), a marked osteoporosis and osteopenia (Fig. 17), with consequently increased bone fragility, multiple Harris lines (Fig. 16), delayed epiphyseal closure (except of the proximal humerus and the distal femur which often show premature and irregular fusion of the growth plate that produces shortening and deformity) and a general reduction of growth which can lead to dwarfism (Canci 2005, Ortner 2003).

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\(^6\) Phenomenon most pronounced in the femur.
Unlike sickle cell anemia, pure thalassemia creates no circulatory obstruction and doesn't have an increased rate of osteomyelitis (Kalifa and Mergouz 2004).

At last, children's limbs usually show enlargement of metacarpals, metatarsals and phalanges with diagonally crossing trabeculae and reticulated thin cortices, leading to a honey-comb pattern of hand and foot short tubular bones (Ortner 2003).

Current treatment consists in regular periodic blood transfusion, iron chelating therapy to remove iron stores from the body (which is caused by over time transfusion), and splenectomy if splenomegaly is present. The only cure is bone marrow transplantation (Healthenciclopedia).

Bones in β-Thalassemia (Ortner 2003, Ortner 2003, Canci 2005)
Also the homozygous form for α-thalassemia exists. But, luckily, this disease is extremely rare. It results in a severe form of anaemia that affects the foetus very early and prevents it to survive: usually, there's a high incidence of miscarriage during gestation or death at birth. Children affected are edematous (Fig. 18) and have little circulating hemoglobin; whole hemoglobin is made up of γ chains, and is called "hemoglobin Bart's (Wikipedia).

Figure 18: child affected by α-thalassemia.

Where Thalassemia Comes from?
The gene of thalassemia is not evenly distributed among peoples. For example, the gene of α-thalassemia is more distributed in people living in South-East of Asia, Malaysia and South of China, whereas that of β-thalassemia is more frequent in people from Mediterranean, in particular Italians and Greeks.

The first one who recognised and described β-thalassemia, distinguishing it from other kinds of anemia, was Thomas B. Cooley, an American doctor, in 1925. In his publication "Splenomegaly in children with Anemia and Peculiar Bones Deformation” he described the peculiar symptoms of the disease, especially the skull modifications and the osteoporosis that affected long bones, and maintained that it affected only the descent of Mediterranean immigrant. Due to Cooley's pre-eminence in the diagnosis and description of β-thalassemia, the disease is also called “Cooley's Anemia”. Instead, the term “Thalassemia” was coined only in 1933 by G. Whipple and W. Bradford; it derives from the greek “thalassa”, which means the sea and “-haima”, which is the blood; so, its meaning is “sea in the blood”. But for Greeks the “thalassa” was the Mediterranean Sea, so “Thalassemia” also conveys the idea of Mediterranean in the blood or, better, the idea of anemia coming from the Mediterranean Sea (Wikipedia).

John Lawrence Angel, a British anthropologist, maintained the Greek origin of thalassemia. During he’s research on skeletal remains coming from Mediterranean region (1927-1977), Angel analyse 2334 specimens coming from several archaeological sites of Eastern Mediterranean (in particular from Turkey, Greece, Cyprus and Morocco), belonging to a period from the Upper Paleolithic to modern age. He used the bone modifications, in particular the porotic hyperostosis of the skull, as a marker of thalassemia, and, on this basis, started searching the way of apparition and diffusion of malaria and thalassemia (landola7).

The connection between thalassemia and malaria is well known: since the malarial Plasmodium cannot reproduce into the abnormal RBCs of thalassemia, this disease represents a protection against malarial infection. That's the reason why the thalassemia gene could thrive in areas full of malaria (landola, Ortner 2003, Wikipedia).

7 www.demarchi.org/thalassemia.htm
If we look at the map of distribution of β-thalassemia, we can see that the areas affected are the same affected by malaria (Fig. 19). So, Angel was persuaded that, to see the historical birth and diffusion of thalassemia, he had to search its marks in the areas already affected by malaria.

The history of malaria is very ancient: it began 60 Mya with the apparition of quartana’s *Plasmodium*. But, probably, it is only during Paleolithic that the vector, the *Anopheles* mosquito, appeared. Anyway, it seems that during all the Paleolithic it was a relative rare disturb. Angel maintained that malaria started its diffusion during Neolithic, when a series of climatic and social changes (e.g. glacier melting, birth and diffusion of agriculture, sedentary life in fertile plains, growth of population) led to a rapid and extensive spread of lots of infection (Alciati et al. 1987, Ortner 2003).

According to this hypothesis, the spread of malaria was strictly connected to the diffusion of agriculture from East to West. Before reaching the Western Europe, agriculture spread in Greece during 4th millennium BC; maybe, during that period Greece was one of the most full of malaria region of Mediterranean (landola). Angel was convicted that β-thalassemia must have originated just in such conditions, in Greece during Neolithic. Then, it would have spread with move of population, contacts and trades. As proof of his theory, Angel used the great percentage of thalassemia’s marks (the porotic hyperostosis) that he found in skeletal specimens coming from Nea Nikomedia, a marshy site on Macedonian coast: 60% of those specimens showed evidence of porotic hyperostosis (while in Near East he founded lower percentage: 50% in Catal Hüyük and only 9% in Cyprus; landola, DeMarchi).

Truly, probably malaria had been a contained phenomenon in Greece till the Archaic Period, when an agriculture reform made cultivation become extensive, and the need of fertile and marshy plain led to a recrudescence or to the first real spread of malarial infection. This fact is confirmed by the Solon Reform (VIII century B.C.), who ordered a land reclamation to solve the diffusion of intermittent fevers. It is just during that period that Greek colonists started establishing colonies around the Mediterranean Sea (landola, DeMarchi).

Ezio Silvestroni and Ida Bianco, the most important researchers on thalassemia in Italy, maintained that malaria arrived Italy during VIII-VII century BC, imported by people coming from the East, maybe Phoenicians and Lyds who had several trades with Italian populations; the unexpected decline of many flourishing Italian cities and populations during Iron Age (e.g. Sardinians, Etruscan, cities like Poseidonia, Selinunte, Adria and Spina) has often been related to malarial epidemic (landola, DeMarchi).

They were also convicted that thalassemia arrived in Italy with Greek colonists soon after the recrudescence of malaria in Greece (Archaic Period8) founding a fertile territory to spread because of its protection against malaria. That’s the reason why, as Silvestroni and Bianco noticed, the areas of most diffusion of the disease in Italy are the same that had been heavily affected by malarial infection in the past (Fig. 20), and those of Greek colonisation: in particular the South of Italy (Campania, Puglia, Calabria), Sicily, Sardinia and the Upper Adriatic (Arzisanrovigo).

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8 See infra.
In particular, during their systematic research on actual diffusion of thalassemia in Italy, Silvestroni and Bianco recorded a very high percentage of thalassemia mutation in the blood of people coming from the area of Po River’s delta (Upper Adriatic): 10% in Ferrara, 16% in Codigoro and 12% in Pomposa. These cities in the past were in direct connection to the important Greek emporium of Spina through an ancient branch of the Po River. Spina emporium in antiquity, from 6th to 3rd century BC, gave hospitality to two nucleus of different populations: one Etruscan and one (maybe the most numerous as the graves’ stores suggest) Greek. The same situation was found also for the town of Adria, another ancient emporium for trades between Greeks and Etruscan (Iandola, DeMarchi).

The connection between the large diffusion of thalassemia in the area of Po River’s delta and the Greek population living in Spina had been maintained also by E. Benassi and A. Toti, two anthropologists from Ferrara who studied several osteological material coming from the first excavation of Spina necropolis (Benassi and Toti, 1957: Observation on bone found in excavation of the necropolis of Spina: confirmation of Greek racial origin of thalassemia; Benassi and Toti 1957, Iandola, DeMarchi, Fig. 21).

Really, now is known that the genetic mutation of β-gene actually present in that area is too recent to have been introduced by Greeks during Iron Age colonisation, going back to at about 70-80 generation ago.

Probably, as Silvestroni and Bianco sustained, such evidence must be connected to a new wave of β-thalassemia coming from the East of Mediterranean during Middle Age, when Venice Republic had lots of trade with Byzantines and Turkish people (Iandola, DeMarchi). But, the first penetration of the disease must really be related to the Greek colonisation during Iron Age (Arzisanrovigo).

Nowadays it is known that there are lots of different mutations that can affect β or α genes and lead to thalassemia; maybe the origin of the disturb is not unique, but different mutations appeared independently through the world. In particular, now is known that β-mutation present in Asia is different from that of Europe, and that its apparition in South of America probably depends on an independent mutation after the introduction of malaria during Bronze Age or during trades of slaves in 900 A.D. (Badens et al. 2000, Iandola, DeMarchi).
But, most important, now is known that porotic hyperostosis is not the characteristic mark of β-thalassemia, as Angel thought, but is an unspecific indicator of stress that can be present in several diseases (Aufderheide 1998, Ortner 2003).

**How to Discriminate Beta-Thalassemia from other Diseases?**

Considering that porotic hyperostosis is an unspecific indicator of stress not only present in beta-thalassemia, a macroscopic and radiological analysis (the normally used) on the specimens are necessary but not sufficient to diagnosticate with certainty the presence of the disease, and other elements also must be taken into account (Schulz 2003).

In fact, porotic hyperostosis can be due to several factors, is strictly connected to diet⁹ and can be present in lots of diseases, such as congenital and iron deficiency anemia, metabolic disorders like rickets, scurvy, osteomalacia, osteopenia, infections and diarrhoeic episodes.

So, the question is: is it really possible to discriminate β-thalassemia from other diseases and other kinds of anemia? And if it is, how can do it?

First of all, it’s necessary to try to reconstruct the socio-economic level of the population under study, what its diet was, what its habits, what the environment where it lived, what diseases and infections and what kind of parasites were presents. The paleoclimatic and archaeological reconstruction is really useful, in particular when it allows to know what people eat and what diseases did it have, if the diet was poor of Iron and oligoelements or if malaria or diarrhoeic episodes were presents (Alciati et al. 1987, Borgognini-Tarli and Pacciani 1993).

Then, the macroscopic examination remains the principal analysis that must be done on the specimens. But it must be done taking into account particular evidences that can suggest the presence of β-thalassemia: a short life at death (in archaeological specimens no treatment was present to try to lengthen life), the presence of a marked porotic hyperostosis on the skull, the typical hair-on-end pattern with the roughening trabeculae re-arranged in radial pattern and the marked erosion of the external cranial surface; the characteristic odontofacial modifications (cheekbones and maxilla hyperplasia, malocclusion and overbite) which lead to rodent’s aspect of affected children’s face; the possible presence of the Erlenmeyer-flask deformities on the metaphysis of long bones.

This kind of examination is easy to be done on complete skeletons, but, unluckily, often specimens are fragmentary. In this case, some technique can help us.

First, it is suggested to use radiology to see more clearly the hair-on-end pattern on the skull and the bone marrow expansion on the post-cranial, with thinning of the cortices and the eventual presence of osteopenia (Schulz 2003).

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⁹ In particular, diets with scarcity of vitamin D and oligoelements as iron, high use of maize (as in South of America) or beans (as in Italy during Iron Age), the malnutrition of the mother during pregnancy and a premature birth, can determine bone modifications similar to those of thalassemia (Ortner 2003).
The application of the immunologic method would be the most useful technique, allowing to observe directly the specimen’s haemoglobin. Unluckily, it leads not always to the wished results.

A very useful technique for a paleopathologist is now the microscopic analysis. It is a bit invasive technique because it is necessary to cut a thin ground section of the bone under examination to analyse its structure on light microscopy (especially with polarized light), micro-radiography or/and Scanning Electron Microscopy (SEM), but such techniques can make a differential diagnosis on dry bones, especially in case of diseases that affect the microstructure of the cortical of long bones and the cancellous bone substance of the skull (e.g. anaemia, scurvy, rickets, meningitis, osteomyelitis, etc); they allow more reliable results than with macroscopic observation, radiology, and also Multiple Helical Computed Tomography (MSTC; Schulz 2003).

Conclusions
Surely, the most entrusting diagnosis of beta-thalassemia is that made taking into account the results of all diagnostic methods listed before10(Schulz 2003).

But, as they often are very expensive, it is not always possible to apply all of them on all the specimens. We want to suggest here to try to have at least a sample detected with such techniques when the presence of β-thalassemia is supposed. Without it, the paleopathologist can only maintain the presence of porotic hyperostosis, and not that of thalassemia.

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10 See supra.
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