

Erythrocyte phenotype in a pregnant woman of Sri Lanka. Description of the case and complications related to communication problems

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Abstract

Background. The Bombay phenotype is a rare genetic trait which is characterized by the absence of A, B and H antigens on red cells as well as in body secretions. The serum shows the presence of antibodies against antigen H. Patients with this rare blood type are not easily transfusable. We had observed a woman aged 18, at the 20th week of pregnancy, native of Sri Lanka, with an IgG and IgM class anti-H. We report the case and the clinical issues arisen.

Materials and methods. The determination of ABO, Rh[D] group, the indirect anti-globulin test (IAT) were performed in tube techniques and in neutral gel microcolumn. Detection for antibodies was performed using ID-Card LISS-Coombs microtubes, in solid phase and with tube techniques. For molecular analysis, the *FUT1* and *FUT2* genes were sequenced using BigDye terminator v1.1. The study of *FUT2* gene was performed after extraction of mRNA using Qiagen kit RNase and then reverse-transcribed into cDNA.

Results. The Bombay phenotype was confirmed by serological and molecular analysis techniques. The patient, in collaboration with a cultural mediator, was informed of her immunohaematological condition and a program of assistance was proposed to her. Unfortunately the patient did not return for the next visit, despite of a telephone reminder. During childbirth a haemorrhage occurred and a request of compatible blood for an urgent transfusion arrived at our transfusion service. Fortunately, the haemorrhage was arrested and the patient didn't need to have any transfusions.

Conclusion. This case emphasizes the need for an efficient management of rare blood types that are more and more frequent as a result of migration. It is necessary to organize, in strategic points of the national territory, reference centres with better diagnostic capabilities and implement freezing of red blood cells with rare phenotype for diagnostic and therapeutical use. Communication issues are as well important in dealing with this emerging phenomenon.

Key words

- Bombay phenotype
- FUT1 and FUT2 genes
- blood

INTRODUCTION

The name Bombay, in the world of blood transfusion, immediately evokes a rare phenotype of red blood cells that recalls the difficulty in finding compatible blood [1]. This phenotype finds its maximum concentration in the Indian city of Mumbai, previously called "Bombay"

[2]. We observed a pregnant patient, carrier of this phenotype, and in this paper we describe the case and the lessons that have emerged from it.

The antigenic molecules A and B are generated by the addition of a specific sugar (N-acetylgalactosamine and galactose respectively) at the end of the chains of

O-glycan and N-glycan that branch off from the membrane proteins and lipids of red blood cells and many other cells [1]. In order to perform this addition the two specific glycosyl-transferases, need to interact with a molecule of fucose in the position $\alpha(1-2)$ of the pre-terminal galactose of the chain. This also applies to the glycoproteins and glycolipids which are secreted from exocrine glands and from endothelial cells in plasma. The fucosyltransferases responsible for this glycosylation are the products of two genetic loci: *FUT1* and *FUT2* [3, 4].

FUT1 is active in the erythroid and other lines and generates a specific fucosyltransferase for Type2 chains present in these cells. *FUT2*, instead, is active in the exocrine and endothelial cells of the vessels, acting on the fucosylation of Type1 chains in the glycolipids and glycoproteins produced by these cells for organic secretions and plasma. The wild gene, suitable for the synthesis of effective transferases at the *FUT1* locus is indicated by *H*, the set of ineffective genes is defined as *h*. At the same time, in the *FUT2* locus, genes are indicated respectively, with *Se* if they are effective and with *se* if ineffective. The presence of only one effective gene is sufficient to produce fucosyltransferase [3, 4]. The *h* gene is extremely infrequent in the global population, the rare homozygotes *h/h* have a frequency of 0.0004% (1/250 000). As previously mentioned, there is a niche of greater expression on the western coast of the Indian subcontinent, where the city of Mumbai has the highest frequency, about 1/10,000 inhabitants. Individuals with the pair of allele *Se/Se* and *Se/se* are defined secretor, while non-secretor subjects are defined by the pair of allele *se/se* where they have a global frequency of around 22% [4, 5].

At the base of the Bombay phenotype, whose symbol is O_h , there is a double homozygous condition *h/h* and *se/se*, so individuals with this genotype will not produce the H molecule and therefore their red blood cells do not exhibit A and B molecules [4, 5]. The absence of substance H in O_h , leads to immunization against this substance present in the sugary wall of the bacteria that colonize the large human intestine: these antibodies, generally IgM, have a wide spectrum of thermal activity, and they are capable of triggering a serious intravascular haemolytic transfusion reaction [6, 7]. An anti-H antibody reacts with all cells except O_h . Individuals with genotype homozygous *h/h*, coupled with the presence of a gene *Se*, result in a strong reduction of H, A and B antigens on the surface of red blood cells, but not their absence, given only by plasma absorption. No anti-H antibodies are formed. This phenotype is called para-Bombay [4].

SUBJECT, METHODS AND RESULTS

Subject

P.R.P.M., a pregnant woman at the 20th week of pregnancy, aged 18, originally from Sri Lanka, underwent the determination of the ABO, Rh[D] group and the indirect antiglobulin test (IAT) at our transfusion service. There was a discrepancy in cell and serum ABO grouping test, and reaction with all three cells test in IAT: this led to the suspicion of a rare blood type.

Methods in serological analysis

The ABO phenotyping was carried out by the standard forward grouping test by using commercial antisera anti-A, anti-B, anti-AB, anti-H (Antisera Immunodiagnostica GmbH). Reverse grouping was performed using A1, A2, B and O cells 3% in Saline 0.9% in tube techniques and in neutral DiaMed microcolumn.

The research for antibodies was performed using ID-Card LISS-Coombs microtubes containing anti-IgG and anti-C3d within the gel matrix and neutral, at 37 °C (DiaMed with 3 + 11 cells test untreated and 11 also treated with papain), in solid phase at 37 °C (Capture Ready ID, Extended I and II, Immucor) and with tube techniques at 20 °C (two Panel Twenty Immucor). Elution with acid glycine was performed using Red Cells Elute by Lorne Laboratories LTD.

Methods in molecular analysis

For molecular analysis, the *FUT1* and *FUT2* genes were sequenced using BigDye terminator v1.1 (Thermo Fisher Scientific), using the primers described in the literature [8, 9].

The study of *FUT2* gene was performed after extraction of mRNA using Qiagen kit RNase and then reverse-transcribed into cDNA. The same procedure and the PCR technique were performed on two control samples using the same primers.

RESULTS

The typing of the patient's red blood cells with anti-A, anti-B and anti-AB showed no reactivity, addressing the diagnosis towards group O. Reverse grouping was performed to identify ABO agglutinins, with known pooled A1, A2, B and O cells: the sample showed agglutination with homogeneous score 4+ with each of them. The IAT was positive showing agglutination with score 4+ each of the 3 cells test. The differential diagnosis was between a Bombay phenotype and an autoimmune disease supported by an IgM antibody. DAT (Direct Antiglobulin Test), the self-control that was executed at 37 °C and 20 °C and typing of the red blood cells using the anti-H lectin of *dolicus biflora*, were negative (as shown in Figure 1). At this point the suspected diagnosis of Bombay phenotype was forcefully supported: the

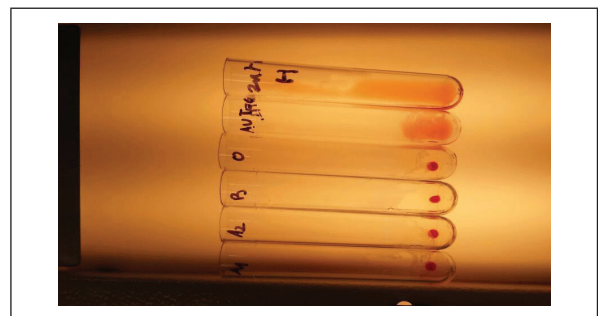


Figure 1

ABO reverse grouping test: the sample shows agglutination with homogeneous score 4+ with each of them. The self-control and typing of the red blood cells using the anti-H lectin of *dolicus biflora*, are negative.

antigenic structure of the red blood cells, the specificity of the antibody present in the serum, and the gene structure of the *H* and *Se* locus were thoroughly studied [10, 11]. Informed consent for molecular analysis was obtained from the patient before collecting her blood sample. Genomic DNA was prepared from peripheral blood leukocytes by standard procedures. The PCR product of both *FUT* genes was directly sequenced. The sequencing of the entire coding region of *FUT1* showed a mutation T725G (as shown in Figure 2) and the sequencing for that of *FUT2*, a 10 Kb deletion, both in homozygous condition. This genotype has been described as a characteristic of individuals with non-secretor Bombay phenotype [4, 12].

The patient, in collaboration with a cultural mediator, was informed in detail of the two risks associated with his immunohaematological condition:

- the risk of foetal and neonatal haemolytic disease (EDFN) given the presence of an important IgG component of anti-H;
- the risk of absence of compatible donor blood for transfusion in the event of post-partum haemorrhage or any pathological event requiring transfusion therapy [13-16].

Unfortunately the patient did not return for the next check, even after a telephone reminder. The patient was hospitalized at the end of the expected period of pregnancy, without advising either our transfusion service or the centre for pregnancies at risk. During childbirth a haemorrhage occurred and a request of compatible blood for an urgent transfusion arrived at our transfusion service. Fortunately, the haemorrhage was arrested and the patient didn't need to have a hysterectomy. The new-born was born healthy and with O, Rh[D] group. The patient showed during her pregnancy poor compliance that led to the inability to carry out pre-deposit and implementation of planned interventions to prevent and counteract possible adverse events related to the case. Events that, in fact, occurred during childbirth. It is possible that this is linked to the cultural background of the patient with behaviour and traditions related to childbirth, which do not provide medical support and hospitalization.

DISCUSSION

In this study, a case of non-secretor Bombay phenotype, diagnosed in our transfusion service, was reported. This genotype is relatively common in the south-

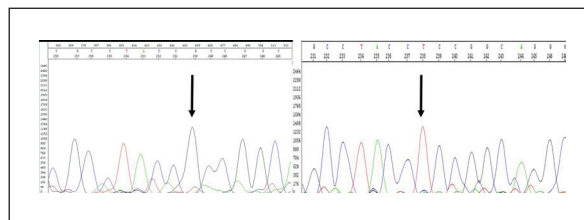


Figure 2

Sanger sequencing for evaluation of SNP presence in *FUT1* exon 4, primer pair 3. The arrow shows the SNP (Single Nucleotide polymorphism) sequence related to L242R (c. 725 T>G). The panel to left shows the SNP homozygote in sample by direct sequencing. The Panel to right shows the wildtype sequencing of a control DNA.

east area of the Indian sub-continent, but in Italy it is almost inexistent and is defined as from a "rare group". Ongoing migratory processes, cause an increased frequency in Italy of people with red blood cell phenotypes present mainly or exclusively in non-caucasian populations. These processes have become more frequent and significant and they are having an important impact on the ethnic composition and genetic-antigenic characteristics of the Italian population even more rapidly than we may have thought, taking into account only the "number" of immigrants present [16]. The increasing immigration present in Italy implies the progressive development of a multiethnic society, in which a very high number of patients from different ethnic groups, are from rare blood groups. Most foreigners come from Central and Eastern Europe, North and Central Africa, some Asian countries and Central and South America.

In this period, in fact, the low rate of fertility of the indigenous Italian population, is counteracted by the relatively high birth rate among immigrants, especially those from Africa, Asia and Latin America [17]. In our region, in the last three years, with an immigrant population of around 10%, the percentage of infants with foreign parents is 30%, of these, about 10% have only one foreign parent as shown in Table 1 and Table 2. We have passed from one multi-ethnic reality, but essentially stable for centuries, to a new multiethnicity. This presents a new challenge for our transfusion system, we can accept and address this challenge by borrowing suggestions from countries that have experienced this situation before us [18].

Table 1

Children born to the Careggi's Hospital (Florence, Italy) by parents with different nationalities

| Years | Births at Careggi's Hospital, years 2014, 2015 and 2016 | | | | | | | | | |
|-------|---|------|----------------------|------|-------------------------------|-----|-------------------------------|-----|-------------|--------|
| | Both Italian parents | | Both foreign parents | | Mother foreign Father Italian | | Mother Italian Father foreign | | No response | Total |
| | N | % | N | % | N | % | N | % | | |
| 2014 | 2577 | 71.0 | 681 | 18.4 | 218 | 5.9 | 152 | 4.1 | 72 | 3700 |
| 2015 | 2526 | 70.5 | 718 | 19.8 | 262 | 7.2 | 79 | 2.2 | 38 | 3623 |
| 2016 | 2430 | 69.6 | 675 | 19.2 | 274 | 7.8 | 113 | 3.2 | 32 | 3524 |
| Total | 7533 | 70.4 | 2074 | 19.1 | 754 | 7.0 | 344 | 3.2 | 142 | 10 847 |

Table 2

Children born in Tuscany Region (Italy) to parents of different nationalities

| Years | Births in Tuscany, years 2014, 2015 and 2016 | | | | | | | | | |
|-------|--|------|----------------------|------|----------------------------------|-----|----------------------------------|-----|-------------|--------|
| | Both Italian parents | | Both foreign parents | | Mother foreign Father Italian | | Mother Italian Father foreign | | No response | Total |
| | N | % | N | % | N | % | N | % | | |
| 2014 | 19 746 | 70.9 | 5571 | 20.0 | 1962 | 7.0 | 556 | 2.0 | 1180 | 29 015 |
| 2015 | 18 939 | 70.9 | 5330 | 20.0 | 1986 | 7.4 | 461 | 1.7 | 965 | 27 681 |
| 2016 | 17 994 | 69.1 | 5495 | 21.1 | 1992 | 7.6 | 572 | 2.2 | 1314 | 27 367 |
| Total | 56 679 | 70.3 | 16 396 | 20.3 | 5940 | 7.4 | 1 589 | 2.0 | 3459 | 84 063 |

In many western countries the main transfusion organizations have promoted the creation of rare group donor registers or banks, coordinated by a central immunohaematology laboratory. This has been established in the European Union with the National Frozen Blood Bank (UK), the Frozen Blood Council (EU) and the BNSPR Banque Nationale de Sang de Paris and in the United States with the New York Blood Center, the ARDP American Rare Donor Program Philadelphia and in the eastern countries The Shanghai Blood Center and the Japanese Red Cross.

In Italy, only the Lombardy region in 2004 established the Bank of rare blood group components – Centro di Riferimento della Regione Lombardia (Lombardy Region Reference Center) at the Centro Trasfusionale Fondazione IRCC Ca' Granda (Transfusion Center of the IRCC Ca' Granda Foundation) (Ospedale Maggiore Policlinico) of Milan, which has a register of donors and a supply of (frozen) blood from rare groups.

This bank is essential for managing requests for blood in cases of complex immunization. By “rare blood” it is understood a type of blood that has a combination of uncommon antigens. A donor is defined as from a “rare group” when his/her antigenic structure is found in at most 1 subject for every 1000-5000 examined. The negative subjects for high incidence antigens, defined as carriers of “extremely rare” groups, are found, however, with an even lower frequency (<1:5000). These blood groups do not pose health problems but underscore evident difficulties in the management of transfusions. In such cases it is obviously very difficult to find blood units suitable for transfusion: the availability of a “Rare Group Donor Register” permits the convening of the appropriate donors at the time of need for transfusion of the patients described above, in this way the establishment of a rare unit frozen blood unit bank allows for the creation of stocks necessary to respond to the transfusion emergencies that may arise.

In Italy, blood donors are still almost all of Italian origin and as a result, there is great difficulty in finding blood bags from rare groups. In Tuscany, even if there is no Rare Blood Bank, such as the one in Lombardy, the Avis Regione Toscana in collaboration with the University of Pisa, has at least promoted a series of initiatives aimed at understanding how the communities of foreign immigrants in Tuscany relate to the donation of blood, on the basis of the relative peculiar cultural assumptions and models of citizenship. These campaigns

to raise awareness highlight the need to implement targeted strategies to encourage donations from ethnic minorities and attempt to overcome cultural barriers as well as promote an increase in social solidarity.

In order to facilitate and thus obtain effective results from raising awareness for donations by foreigners, it would be fundamental to import to the various Italian regions the Lombardy model as mentioned above, where a “Rare Groups Blood Bank” was created as well as a “Donor Register”. The National Blood Center could be designated for coordination among the various regional structures, to make it possible to retrieve the blood of rare groups even beyond the territorial boundaries of the Regions.

This would represent a significant step forward in the solution of transfusion problems related to the rarity of blood groups in the area and would also allow a better and more effective management of human and non-human resources engaged in the sector, as well as a rationalization of costs [19-21].

CONCLUSION

The case described has given us many relevant points of discussion. It highlights how the National Health System does not take charge of solving the problems that result from the presence in a modern society of a growing number of people with different origins and cultural characteristics thereby causing the dysfunctions mentioned in the present study which are inherent to structural shortcomings as well as communication problems, that are destined to occur over and over again. In fact, the State should be the first and most important source of communication with all its citizens, as well as patients in healthcare facilities, including and above all through pre-established structures, organized and equipped with the necessary expertise. A centralized structure, holding donor registers and blood stocks of rare groups, with decentralized and coordinated branches, would facilitate health and hospital units present on the territory with the management of the most complex cases from the point of view of transfusions. This should be accompanied by an appropriate implementation of information campaigns aimed at encouraging the donation of blood components by people from all the different ethnic groups, who live together in the same territory.

At the moment, in the sectors in which the protection of an individual's health is left to the mere willing-

ness of health personnel, in the absence of guidelines and structures, such as those indicated and hypothesized above, the management difficulties in assisting patients with rare blood groups, can even become insurmountable.

A State that does not equip itself with the necessary and appropriate tools to create effective communication with all its citizens, and therefore including those who need special health care/treatment, to make them aware of their pathophysiological situations, associated risks as well as the preventive and therapeutic possibilities available to them, cannot demand from them the

active participation that is necessary for achieving the “health” objective, common to all.

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Conflict of interest statement

The Authors declare no conflicts of interest.

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